

# Progress in Eye and Vision Research 1999–2006



U.S. Department of Health and Human Services  
National Institutes of Health  
National Eye Institute

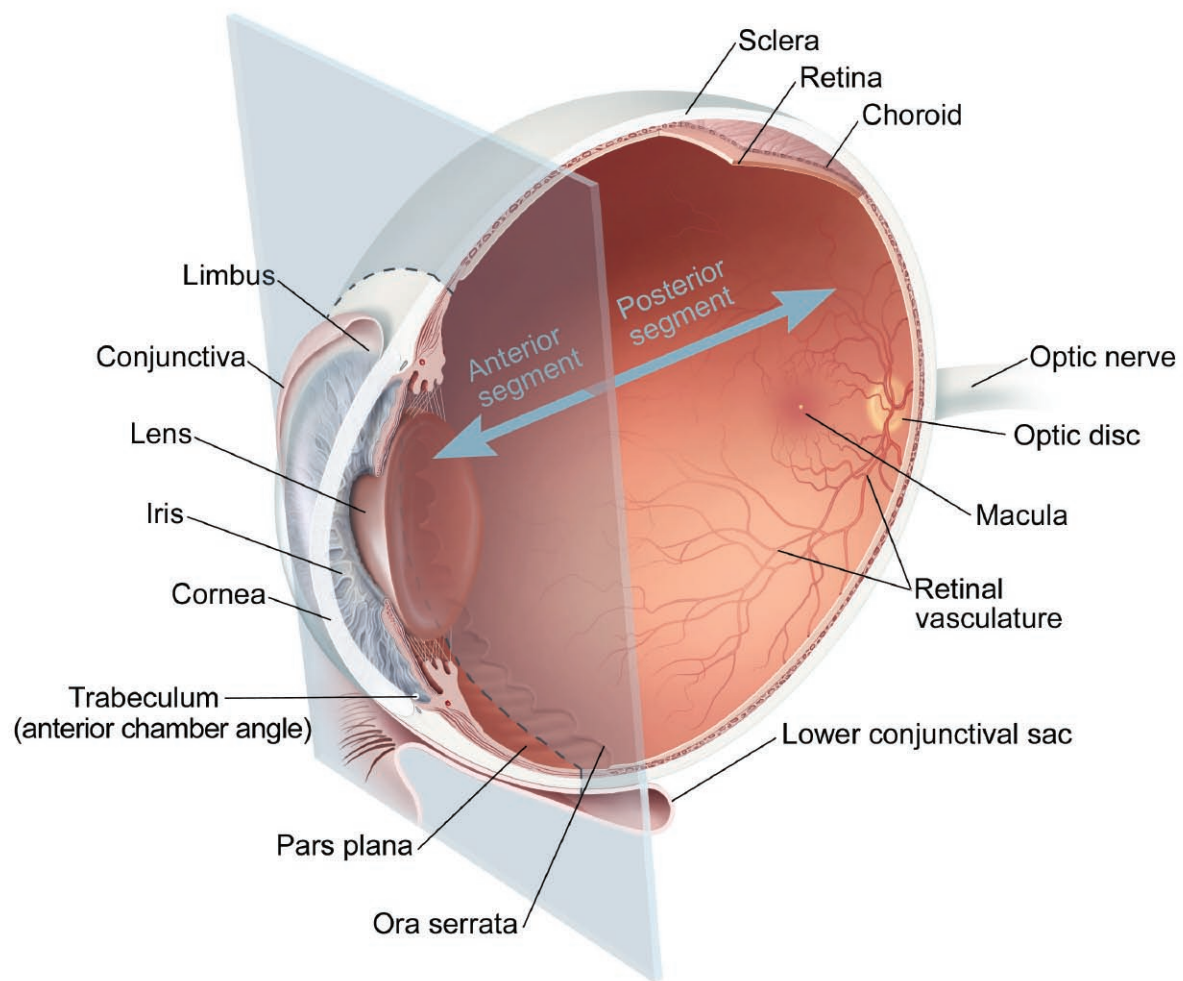




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## Foreword

Medicine has entered an exciting new epoch with enormous potential to understand and develop treatments for even the most intractable diseases. On the laboratory front, we have learned to sequence the entire human genome. The recent availability of sophisticated genetic tools now enables researchers to quickly scan the human genome to discover the genetic basis of even the most complex diseases. Each new gene discovery tells us more about how cells function within the body and how genetic alterations cause disease. The knowledge gained from this process allows medical scientists to develop rational treatments that address the root biological causes of disease. Great progress has been made, but much work still remains.

The United States Congress recognized this unique point in our history and doubled the budget of the National Institutes of Health (NIH) between 1999 and 2003. These budget increases provided the NIH with new resources to realize the vast potential that exists to cure disease. The investment is already paying substantial dividends.

Vision scientists, supported by the National Eye Institute (NEI) of the NIH, used these augmented resources and delivered stunning research advances to the American people. In less than a decade, NEI-supported laboratory scientists have developed new treatments with the potential to prevent vision loss and, in some cases, restore sight for common eye diseases such as macular degeneration, glaucoma, diabetic retinopathy, amblyopia, retinopathy of prematurity, uveitis, and many rare but equally as devastating diseases. These investigational therapies are now entering clinical trials and some will become treatments for the millions of Americans with eye disease.

This document was prepared to convey the width and breadth of vision research progress that has occurred in concert with these historic budget increases. As Director of the National Eye Institute, and a physician and scientist who has devoted my professional life to alleviating suffering from eye disease, I am committed to ensuring that the portfolio of NEI research is leveraged as quickly and efficiently as possible. As you read this document, I hope that you will gain a sense of excitement about the possibilities this work holds ultimately to rid the world of blindness and eye disease.

Paul A. Sieving, M.D., Ph.D.  
Director  
National Eye Institute



# Introduction

## The Gift of Sight

The human visual system outperforms the best cameras, offering stunning clarity and vivid color with features like auto focus, built-in light meters, automatic f-stops, and wide angle and telephoto lenses. A lightening fast image processing unit in the brain, called the visual cortex, develops and analyzes the steady stream of images that enters our eyes to reveal the richness of the world in real time. The visual system recharges while we sleep and flicks back on in a literal blink of an eye.

But vision is much more than the complex biological machinery that records our surroundings. It is the primary sense we rely on in our daily lives. Fully one-third of the cerebral cortex—the part of the brain that carries out our higher functions—is dedicated to processing the images we see each day. The visual cortex connects to the neural pathways in the brain that govern memory, emotions, decision making, and movement, to inform and orient our consciousness. For example:

**THE WARM FEELINGS WE EXPERIENCE** when returning the smile of a loved one are possible because our visual system connects with regions in the brain that can identify the people in our lives, interpret facial expressions, and elicit emotional responses.

**WHILE READING THIS SENTENCE**, your visual system is linking with areas of the cerebral cortex that decode, remember and comprehend written language.

**THE ARM MOVEMENTS WE MAKE** when reaching for an object are guided by visual processes in the brain that plan each move without ever consciously thinking about it.

**WHEN CROSSING A BUSY CITY STREET**, we depend on visual processes that interpret motion and speed so that we can avoid the oncoming cars and make it safely across the street.

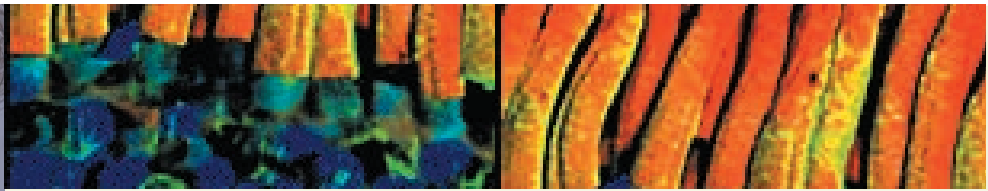
**THE MERE SIGHT OF OUR FAVORITE FOODS** stimulates neurons in the brain that whet our appetite and activate our salivary glands.

The pure pleasure we derive from vision is hard to describe but easily understood. Since the first cave paintings, mankind has been enthralled with the visual arts. When visiting a gallery, we are drawn to the sheer grace of a painting by masters like the 17th century Dutch artist Vermeer. On closer inspection, we notice the streams of light flooding through the window in the painting but can't find any visible brush strokes that reveal the artist's hand. We are awed by Vermeer's ability and the visual experience becomes sublime. We move on to the next painting in the gallery hoping our eyes will be similarly dazzled.



Rijksmuseum, Amsterdam





Whether its watching our children grow, reading a good book, becoming engrossed in a movie, taking in the natural splendor of fall foliage, sinking a 25 foot putt, flying an airplane, or training our sights on the countless other things of our choosing, vision allows us to experience the varied pleasures of human existence.

## The Biology of Sight

The visual system is a marvel of biology. Many of its parts are unique to human physiology. At the front of the eye, the cornea shields dirt and debris from the outside world. The cornea is rich with nerve cells that register pain or discomfort at the least provocation. When a speck of dust or grain of sand lands on the cornea, these pain receptors activate a set of wiper blades and washer fluid, more commonly known as eyelids and tears, to instantly squeegee the cornea clean.

The cornea and the lens are the only tissues in the body without a vasculature. The lack of blood vessels makes the cornea and lens completely transparent so these tissues can focus light on the retina at the back of the eye.

At each tip of the more than 125 million photoreceptor cells that line the retina is a light processing factory called an outer segment. An outer segment is composed of a stack of thin discs that resemble poker chips. These discs contain the molecules necessary to absorb and convert light into an electrical signal. While we sleep, the biologically spent discs at the top of the outer segment are shed and eliminated as waste while new discs at its base are created. An outer segment is totally renewed over the course of 9 evenings, allowing the retina to continually process light during our waking hours.

The constant stream of signals produced by photoreceptors pulsates through a neural network of cells in the retina that begin the complex task of creating a coherent image. These cells, known as bipolar, amacrine, horizontal, and ganglion cells add “date stamps” to chronologically order the signal stream, convey light intensity to recreate conditions of brightness or dimness, and relay color information.

The modified signals are then transmitted, via the optic nerve, to the visual cortex of the brain, where image processing and interpretation occur. The visual cortex is a complex network of 30 or so distinct areas that process different aspects of an image. The first, largest and best understood area is called the primary visual cortex, or V1, for short. It recognizes forms and shapes and is connected with regions of the brain that control eye movements, hold long-term memory, and plan arm movements. Other areas within the visual cortex detect motion, color, depth, and place objects within the visual field. Still other areas analyze an image to interpret its meaning. And so, the images we see in real time are actually first broken down by their constituent parts and later reconstituted. As each second ticks by, the visual cortex is making millions, possibly billions, of calculations to produce images.





## Eye Disease

Such an elegant and complex biological system is prone to a host of diseases and disorders that robs millions of Americans of their vision.

Imperfections in the cornea or lens and even the size and shape of the eye itself affect the eye's ability to focus light appropriately and determine whether the images that reach the retina are blurred. **Refractive error** affects 25 percent of the American population, requiring correction through glasses, contact lenses, or surgery. Moreover, because the cornea is rich with pain receptors, diseases and injuries of the cornea are extremely painful, requiring immediate medical assistance.

For reasons that are not entirely understood, the clear lens in the eye becomes susceptible to clouding, a condition known as **cataract**. It is estimated that 20.5 million Americans over age 65 have either had cataract surgery or currently suffer from the condition. Although cataract extraction is a proven method to restore lost vision, the costs of care place a huge economic burden that is largely shouldered by the Federal government through its Medicare program.

Retinal degenerative diseases such as **retinitis pigmentosa** and **macular degeneration** cause the loss of light-sensing photoreceptor cells. A recent study finds that over 1.8 million older-age Americans are living with severe vision loss from macular degeneration and another 7 million are at risk of losing sight to the disease.

**Glaucoma**, a disease that destroys ganglion cells in the retina, affects over 2 million Americans. African Americans are disproportionately affected with a rate that is three times that of Caucasians.

It is estimated that **uveitis**, an inflammatory disease within the eye of infectious or non-infectious origin, accounts for 10 percent of blindness in the U.S.

**Diabetic retinopathy**, a complication of diabetes mellitus, affects 4.1 million Americans. One in 12 people with diabetes over age 40 has lost vision to diabetic retinopathy.

Many eye diseases and disorders affect infants and young children, leaving them susceptible to a lifetime of blindness or extreme visual impairment. For example, each year, **retinopathy of prematurity** affects thousands of low-weight, premature infants. **Amblyopia**, more commonly known as lazy eye, is a disorder where the brain does not process images from one eye. If left untreated, children with amblyopia lose depth perception. It is estimated that 3 percent of children in the United States suffer visual impairment from amblyopia.

The human toll of blindness and visual impairment cannot be measured by sheer numbers alone. On a personal level, because we rely so heavily on the sense of sight, it is difficult and frightening to imagine living in darkness. This concern is expressed in survey data which find Americans consistently ranking blindness second only to cancer as the most feared malady. Loss of vision threatens our independence. Blindness or visual impairment may cause the loss of our driver's licenses. It may shut us in our homes, disable productive careers, strip us of the hobbies we enjoy, and obscure the faces of loved ones. As mobility and independence vanish in the haze, depression becomes common. In an effort to quantify the scale of human suffering, a recently published study found that patients with vision loss from macular degeneration rated their health-related quality of life in a similar manner to patients with serious medical conditions such as dialysis dependent renal failure or AIDS.



## The National Eye Institute

The National Eye Institute (NEI), a part of the Federal Government's National Institutes of Health, is working to preserve and restore the vision of Americans through medical research. The NEI supports approximately 1600 research grants and training awards made to scientists at more than 250 institutions across the country and around the world. The NEI also conducts laboratory and patient-oriented research at its own facilities located on the NIH campus in Bethesda, Maryland. Almost every major breakthrough in eye disease research has resulted from the support of the NEI through the funding it receives from the Congress.

Over the last decade, we have witnessed a revolution in medical science with the potential to treat and cure even the most intractable diseases, including eye disease. Vision researchers have leveraged the Human Genome Project and other powerful genetic research tools to find alterations in genes that cause blinding diseases. With the genetic basis for many eye diseases now known, cell biologists are studying how genes function in health and disease. As a result, the molecular events that cause many eye diseases are becoming ever clearer, giving us greater insights into treating these diseases.

As life expectancy has increased, neurologic diseases associated with aging—Alzheimer's, Parkinson's, and macular degeneration—have become more prevalent. In fact, many common eye diseases damage the neurons that process light. Neurologic diseases present researchers with vexing difficulties. Neuronal cells are post-mitotic, meaning that they no longer divide and reproduce to replace cells that no longer function. Post mitotic cells must function over an entire lifespan. A loss of any cell type due to disease or injury disrupts the complex neural circuitry that allows us to see. Despite this complexity, NEI researchers are leveraging neuroscience to develop therapies that prevent the loss of neuronal cells and in some cases regenerate new nerve cells.





Genetics, cell biology, neuroscience and many other research disciplines are creating unheralded therapeutic opportunities. For example:

- Using gene therapy, NEI funded researchers have restored sight in dogs born blind with a severe form of retinitis pigmentosa that affects infants. A clinical trial is planned for 2006.
- NEI researchers are testing a drug that blocks the cellular signals that trigger damaging inflammatory responses in uveitis.
- NEI researchers recently discovered a common alteration in a gene called complement factor H (CFH) that accounts for almost half of all cases of age-related macular degeneration (AMD). Discovery of the CFH gene in AMD is a bellwether finding that now allows us to jump start efforts to understand the biological mechanisms that cause the disease and to begin contemplating rational, therapeutic interventions.
- A class of naturally occurring proteins, known collectively as neurotrophic agents, has prevented vision loss in animal models of glaucoma and retinitis pigmentosa. The NEI is sponsoring a clinical trial testing one of these drugs, known as ciliary neurotrophic factor, in patients with retinitis pigmentosa.
- NEI sponsored clinical trials have discovered that early intervention with eye pressure reducing drugs can prevent serious vision loss in patients with glaucoma.
- An NEI sponsored clinical trial, the Age-Related Eye Disease Study, found that a daily regimen of antioxidants can delay severe vision loss from macular degeneration.
- A new computerized risk assessment model developed by NEI-supported researchers proved effective in identifying low birth-weight, premature infants at the highest risk of developing severe vision loss from retinopathy of prematurity. This new model enables doctors to treat in the earliest stages of disease, thereby saving vision.
- NEI clinical trials have established the value of eye patching and drug regimens for amblyopia.
- NEI funded research has discovered new methods of regenerating neuronal cells, giving rise to the possibility that replacement of nerve cells lost to disease is feasible.
- NEI scientists are developing novel prosthetic devices that may restore limited vision to the blind. These devices are being evaluated in clinical trials.



## **THE FUTURE**

Thanks to a very productive period of laboratory research, we are beginning to understand the cellular mechanisms underlying eye disease. The NEI now faces the challenge of developing treatments that address the root causes of disease. This effort, known as translational research, has become a major pursuit of the NEI and NIH. In reading the scientific discoveries described in this document, one can see the boundless possibilities that exist to prevent vision loss and restore sight for the millions of Americans who live with eye diseases.

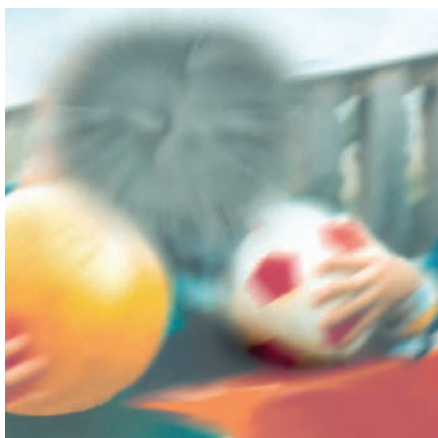


## Advances in Retinal Disease Research

THE RETINA is the complex, light-sensitive, neural tissue in the back of the eye that contains highly-specialized and metabolically active photoreceptor cells (rods and cones). These cells respond to light by emitting chemical and electrical signals. The signals are received by other retinal cells that process and transmit visual information via the optic nerve to the brain for further processing. The choroid is the underlying layer of blood vessels that nourish the retina. The retina and choroid are susceptible to a variety of diseases that can lead to visual loss or complete blindness.



Fundus photo of a normal retina



SIMULATION  
View seen with Age-related Macular Degeneration  
(Normal Vision view on page 8)

## Age-Related Macular Degeneration

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss among persons over 65 years of age, the fastest growing segment of the US population<sup>1</sup>. AMD causes the loss of light sensing photoreceptor cells in the macula, the central portion of the retina that provides us with sharp visual acuity and color vision. In the initial phases of the disease, patients experience trouble reading fine print and seeing in dim light. During the advanced stages, the disease destroys the macula, resulting in severe vision loss and legal blindness. Patients with advanced AMD can no longer read, recognize faces, drive a car, or perform simple daily tasks that require hand-eye coordination. AMD greatly diminishes mobility, independence and the quality of life.

Often, otherwise healthy seniors cannot maintain an independent lifestyle and are forced to seek institutional living alternatives. As the US population ages, the prevalence of AMD is expected to rise sharply, placing ever greater burdens on healthcare and social services. Currently, AMD can neither be prevented nor reversed. However, NEI scientists have made important advances toward treatments and cures for the disease.

### ANTIOXIDANT THERAPY

Previous NEI-supported animal and observational epidemiologic studies have suggested that deficiencies in certain antioxidant nutrients and trace elements may be associated with the development of AMD. Based on these findings, the NEI sponsored the Age-Related Eye Disease Study (AREDS) to evaluate the effects of antioxidants on the development and progression of the disease. The AREDS was a large, multi-center clinical trial involving 4,757 participants, 55 to 80 years of age, in 11 clinical centers nationwide. Researchers conducting the study found that people at high risk of developing advanced stages of AMD lowered their risk of progression by about 25 percent when treated with a daily, high-dose combination of antioxidant vitamins C, E, and beta-carotene, and the trace element zinc<sup>2</sup>. This nutritional therapy represents the first treatment to slow the progression of AMD and delay the onset of severe and debilitating vision loss.

**Based on published prevalence data, an estimated 8 million Americans at least 55 years old are at high risk to develop advanced AMD<sup>1</sup>.** Based on results from the AREDS, 1.3 million of these people would develop advanced AMD over the next five years if no treatment were given to reduce their risk. If this at risk population availed themselves of the AREDS nutritional formulation (vitamins C, E, beta-carotene, and zinc), more than 300,000 would avoid advanced AMD and its associated vision loss over the next five years<sup>3</sup>.

*Based on published prevalence data, an estimated 8 million Americans at least 55 years old are at high risk to develop advanced AMD<sup>1</sup>*

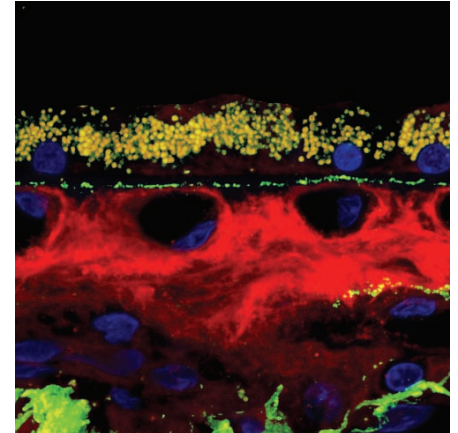


Advances in Retinal  
Disease Research

### GENE DISCOVERY

Late onset, neurodegenerative diseases like AMD are thought to result from the confluence of genetic predisposition and chronic exposure to environmental risk factors. In this scenario, a gene or genes contain subtle variations, known scientifically as polymorphisms, that hamper cellular function but may not result in disease. However, years of environmental insult, such as cigarette smoking, poor diet, and hypertension, further strain the underlying genetic burden to a tipping point that provokes outright disease. On the genetic side of the equation, identifying polymorphisms in AMD and other late onset diseases has been complicated by the fact that traditional genetic research strategies and tools are either inadequate or too cumbersome in their application. The development of more sophisticated genetic tools, such as the International HapMap Project, has enabled scientists to scan the entire human genome more quickly and efficiently. **Using data from the HapMap Project, four different NEI-supported laboratories identified a common variation in a gene called complement factor H (CFH) that accounts for as much as 50 percent of AMD cases<sup>4,5,6,7</sup>.** The CFH protein regulates a specific inflammatory response called the alternative complement cascade that is typically triggered by infectious microbes. Alterations in the CFH gene are thought to poorly regulate this response, leading to chronic, localized inflammation and ensuing damage to cells in the macula and neighboring tissues.

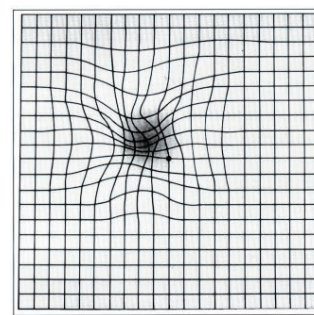
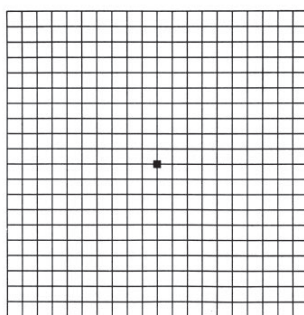
Specifically, it is proposed that functional alterations in the CFH protein result in uncontrolled complement activation over time with subsequent bystander injury to the macula, resulting in its degeneration and an associated loss of vision. The role of CFH in AMD was further strengthened by the finding that macular drusen are associated with a rare kidney disease, MPGN type II, caused by chronic inflammation due to uncontrolled activation of the immune complement system<sup>7</sup>. Drusen are abnormal deposits that form in the macula in association with AMD. Drusen deposits likely interfere with the function of retinal pigment epithelial and photoreceptor cells in the macula, resulting in loss of central vision. Inflammation is thought to play a role in several other common diseases including Alzheimer's disease, Parkinson's disease, multiple sclerosis, atherosclerosis, and stroke. Although the cells, tissues, and molecular events in these diseases are diverse, they may share some common disease mechanisms that present an opportunity to cross-pollinate findings from diverse research areas. Discovery of the CFH gene will also make it possible to develop new animal models and evaluate therapies that control or minimize complement activation.



Cross-section of the retina showing CFH in red



LEFT: Amsler grid as it would appear with healthy vision  
RIGHT: Amsler grid as it would appear to someone with AMD



## GENETIC MUTATION IN MALATTIA LEVENTINESE

One method of elucidating the genetics underlying AMD is to study genes with mutations that cause classically inherited, early onset forms of macular degeneration. These early onset forms share many clinical characteristics of AMD. In a previous NEI-supported study, researchers discovered mutations in the fibulin 3 gene, which cause malattia leventinese, an inherited form of macular degeneration<sup>8</sup>. Like AMD, this disease is characterized by drusen under the retina. Drusen deposits are the first clinical manifestations of AMD. Despite the similarity of these diseases, the fibulin 3 gene has not been implicated in AMD. However, fibulin 3 is part of a recently discovered family of genes that are very similar in structure and function. And so, NEI-supported researchers looked at members of the fibulin gene family in patients with AMD to determine whether these genes might play a role in the disease<sup>9</sup>. Of 402 patients, 7 had sequence variations in the fibulin 5 gene that were not present in age-matched controls who did not have the disease.

Fibulin 5 encodes an extracellular protein that helps form elastic fibers in skin and blood vessels. These fibers are found in Bruch's membrane, which adjoins the RPE. Although fibulin 5 accounts for a small fraction of AMD, this study is one of the first to find genetic sequence variations associated with AMD. Additionally, the study findings suggest that an examination of other genes preferentially expressed in the development and maintenance of Bruch's membrane is warranted.

## RISK FACTORS FOR AMD

Despite its high prevalence, AMD remains a disease with limited treatment options. The identification of risk factors for AMD can provide clues about the causes of the condition; suggest possible new treatment strategies; and provide valuable public health information to lower the risk of developing the disease. The AREDS was in part designed to identify risk factors associated with the disease. The AREDS authors confirmed previous findings that suggested two potentially modifiable risk factors, smoking and hypertension, are associated with the disease<sup>10</sup>. Cessation of smoking and the control of hypertension have wide-ranging health benefits beyond potentially reducing the risk of AMD. For some patients, prevention of vision impairment may be an additional motivating factor to help them modify these risk factors.

## ZINC SUPPLEMENTATION AND EYE DISEASE

An analysis by NEI intramural researchers of data collected from the AREDS established that AMD is associated with increased mortality and that zinc supplementation was associated with decreased mortality<sup>11</sup>. Whether physiologic aging explains the associations between ocular disease and mortality or whether there are factors associated with the development of ocular diseases that are also related to mortality, such as cardiovascular disease, cannot be determined from this study. However, it is possible that factors associated with AMD and cataract development, such as smoking, diet and body mass index, could be responsible for the increased risk of both the ocular diseases and mortality. Based on these and other findings, the NEI study authors recommend that a diagnosis of either cataract or AMD might be one more reason to modify life style habits to promote good health. Additionally, the preliminary association between zinc supplementation and decreased mortality in older Americans provides an important direction for future research.



### RETINAL PIGMENT EPITHELIAL CELL DEATH

The retinal pigment epithelium (RPE), a non-dividing cell layer that lines the neural retina, is integral to maintaining a number of physiologic functions important to vision. The RPE is also thought to be the tissue where AMD manifests. In some cases of AMD, RPE cells die in mass, leading to a form of AMD called geographic atrophy. If scientists could prevent or delay the death of these cells, the severe vision loss associated with geographic atrophy might be ameliorated.

When cells die, they initiate an orderly biological process called programmed cell death or apoptosis, which destroys toxic cell components without damaging neighboring cells. Most investigations of programmed cell death have focused on certain molecules called caspases that initiate damage to DNA within the cell nucleus. Although caspase-dependent cell death plays a role in many cell types, NEI intramural researchers have demonstrated conclusively that the caspase system is not involved in RPE programmed cell death<sup>12</sup>.

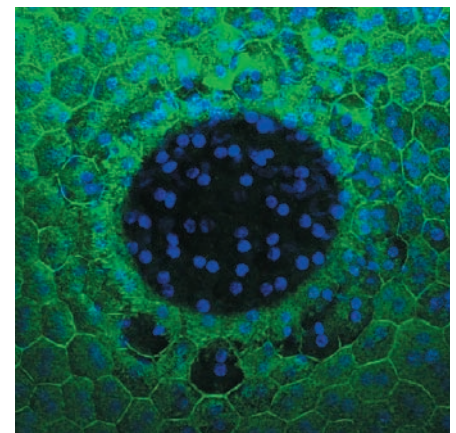
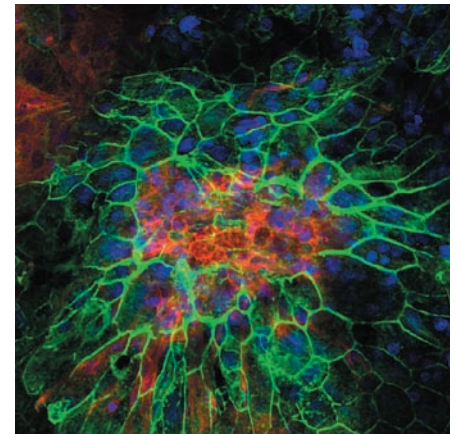
By using a variety of molecular and cellular techniques, they found that a different molecule, apoptosis-inducing factor, was the primary protein involved in RPE cell death. Furthermore, the scientists found that treatment of RPE cells with yet another protein, called hepatocyte growth factor/scatter factor, prevented cell death. This is the first observation suggesting a potential therapy for the geographic form of AMD. Therefore, continuing this research toward developing an effective treatment for this condition would have significant health impli-

cations and would potentially reduce the financial burden of disability costs for these patients.

### NEOVASCULARIZATION

In the later stages of AMD, abnormal blood vessels grow beneath the retina and cause severe vision loss. Owing in part to the lack of an animal model to study the biological mechanisms that spur neovascularization, this critical stage of the disease has not been well understood. However, clinical research has indicated that certain proteins involved in the growth of blood vessels are elevated in patients. Among these proteins, vascular endothelial growth factor (VEGF) is consistently elevated in patients with neovascularization.

For the first time, scientists at the National Eye Institute, using a system to manipulate the genetic expression of VEGF, have been able to cause rats to develop abnormal blood vessels that are identical in location and appearance to those seen in humans afflicted with the disease<sup>13</sup>. Modeling this condition in animals will provide an invaluable research tool to study the causes and to test treatments for this condition. Because the model takes advantage of a stimulus known to occur in the human condition, a more precise understanding of the trigger factors for the growth of the blood vessels will be gained. Subsequently, these trigger factors can then be manipulated through various therapeutic mechanisms that should be directly applicable to patient care for other diseases in addition to AMD.



Images of neovascularization in rodent models



**SIMULATION**  
View seen with Retinitis Pigmentosa  
(Normal Vision view on page 8)

## Retinitis Pigmentosa and Related Retinal Degenerative Diseases

Retinitis pigmentosa (RP) describes a spectrum of eye diseases resulting from genetic mutations that are found in retinal cells. To date, NEI-supported researchers have identified more than 100 genes with mutations causing RP and related conditions. Despite this genetic variation, they are all chronic, progressive diseases that cause the degeneration and death of photoreceptor cells in the retina. These diseases usually present during childhood or early adolescence with night blindness followed by a gradual loss of visual field that leaves patients with very little, if any, central vision. Other retinal degenerative diseases such as Stargardt and Best disease cause the loss of cone photoreceptor cells in the macula, leaving patients with only peripheral vision. Important progress has been made in our understanding of these diseases and offers the hope for future therapies.

### GENE THERAPY

NEI-supported researchers have been evaluating a promising gene therapy treatment that has restored vision in dogs born blind with a severe form of RP called Leber congenital amaurosis (LCA)<sup>14</sup>. Children with LCA are born with severe visual impairment that often leads to complete blindness at an early age.

A gene causing LCA, named RPE65, was identified by NEI researchers in 1997. That same year, mutations in the RPE65 gene were identified as the cause of LCA in Briard dogs. In 2000, a team of researchers injected a single dose of a gene therapy treatment containing the RPE65 gene in three Briard dogs with LCA. Visual evaluation revealed the dogs had significant recovery of vision, allowing them to avoid objects when walking and track movements. The dogs have retained their vision with no sign of complications.

Ever since this breakthrough, the team has been working to gain approval to begin human clinical trials. One of the challenges with this therapy is to determine how late in the disease process one can therapeutically intervene. Although mutations in the RPE65 gene cause severe vision loss, the structure of the retina remains intact for some indeterminate period of time. Eventually, however, retinal cells begin to die and at some point the disease is no longer treatable with gene therapy. Therefore, before a patient could enter a clinical trial it would have to be determined whether his/her retinas were still viable. Using high resolution imaging technology called Optical Coherence Tomography, NEI-supported researchers studied the structure of the retina of patients with LCA and controls without disease to determine whether it was possible to ascertain the health of the retina<sup>15</sup>. Adults with LCA had evidence of thinning in some portion(s) of the retina, indicating that some cell loss had occurred. The study authors found that for the therapy to be successful in adults with LCA, it will be necessary to target surviving tissue in the retina.



### ADULT BONE MARROW STEM CELLS

A recent NEI-supported study found that eye injections of bone marrow derived stem cells prevented vision loss in two rodent models of RP<sup>16</sup>. RP first causes the loss of light sensitive rod photoreceptor cells in the retina. Rod cells provide peripheral and night vision. For reasons that are not entirely understood, the sick and dying rod cells also cause cone photoreceptor cells to die. Cone cells are concentrated in the macula, the center of the retina, and provide the sharp visual acuity that allows us to read, recognize faces, and perform detailed tasks that require hand-eye coordination. As the disease progresses patients lose their central vision, resulting in severe visual impairment or total blindness. **This study raises the possibility that patients could receive an injection of their own bone marrow stem cells to preserve central vision.**

### PHOTORECEPTOR PROTECTION

Phototransduction is the biochemical process that initiates vision. Light strikes and then activates a molecule of rhodopsin, the visual pigment in rod photoreceptor cells, initiating a cascade of events that ends with visual signals being sent to the brain. Activated rhodopsin is recycled back to its normal inactive state through an enzymatic recovery process involving 11-cis retinal, a vitamin A derivative. It is known that light exposure accelerates retinal degeneration in animal models of Stargardt macular dystrophy and some forms of RP. Scientists have proposed that slowing rhodopsin cycling might provide a useful therapeutic strategy for retinal diseases where similar mechanisms of degeneration are involved.

Another derivative of vitamin A called isotretinoin, which is frequently prescribed in humans for severe acne, impairs rod cell function resulting in night blindness shortly after treatment is begun. In a recent research study, isotretinoin had a protective effect in rodents that are susceptible to photoreceptor degeneration due to light exposure<sup>17</sup>. Isotretinoin reduced the rate at which activated rhodopsin interacts with 11-cis retinal in the phototransduction recovery pathway, but does not cause death of the photoreceptor cells. Therefore, slowing of rhodopsin regeneration by isotretinoin may protect photoreceptors from light damage in certain retinal and macular diseases where light exposure is known to accelerate vision loss.



## Uveitis, Immunity, and Infection

Uveitis is a condition in which tissues in the eye become inflamed. Uveitis can be caused by infectious agents, such as viruses or bacteria, or may be of noninfectious origin. Noninfectious inflammation is due to problems with immune regulation. If not properly treated, chronic inflammation causes scarring and leads to irreversible vision loss. Uveitis accounts for an estimated 10-15 percent of blindness in the United States. Understanding these immune and inflammatory processes is an important goal of research supported by the NEI.

### IMMUNOMODULATION IN UVEITIS

Currently, immunosuppressive agents are used to treat uveitis; however, chronic administration of these therapies has many serious and life threatening complications. NEI intramural scientists are working to develop a novel therapy based on reprogramming the immune system, an approach called immunomodulation. Bacterial toxins can alter immune responses when included in small, nontoxic, doses in an immunization protocol. **Using a small dose of cholera toxin, NEI scientists were able to protect a rodent model of uveitis from developing this blinding disease<sup>18</sup>.** This study offers proof of concept that immunomodulatory treatments might be a valuable therapy for uveitis.

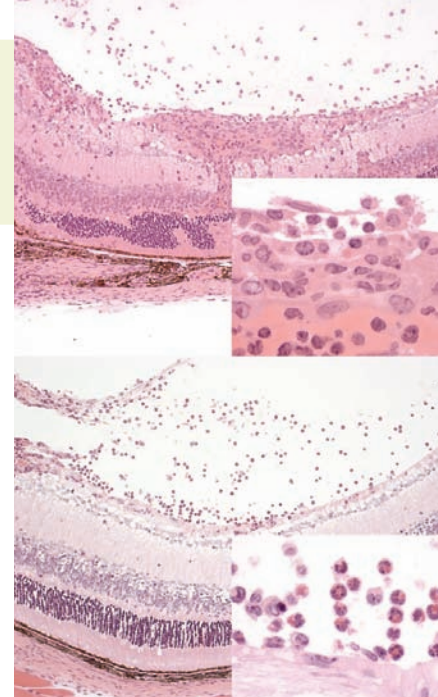
### TARGETING THE CHEMOKINE RECEPTOR

NEI researchers have been studying the underlying molecular cause of inflammation in uveitis to develop more effective treatments for the disease. A major component of all inflammatory responses is the

migration of immune cells into the affected tissue, a process that is mediated and controlled by a family of signaling molecules called chemokines. These molecules activate the immune system by attaching themselves to immune cell receptors on the cells' surface and then signaling other immune cells to invade the target tissue. The immune cells that migrate first into inflammatory sites are called T-helper cells. The migration of these T-cells toward the target site is facilitated by the chemokine receptor designated "CXCR3." Using an experimental system in which T-cells induce ocular inflammation in mice, scientists at the NEI discovered a unique pattern of expression with CXCR3 receptors that facilitates inflammatory responses. Most importantly, NEI researchers found that an antibody that blocks CXCR3 receptors strongly inhibited the development of disease in these animals<sup>19</sup>. The pathogenic process of ocular inflammation in the mouse is very similar to that in humans and, therefore, the new observations made in this study could be applied for the development of more effective and safer treatments.

### MONOCLONAL ANTIBODY THERAPY

In collaboration with researchers at NCI, NEI intramural scientists previously used a monoclonal antibody (daclizumab) as a treatment for experimentally induced autoimmune uveitis in nonhuman primates and found that the treatment had a positive therapeutic effect. Researchers have now reported the results of use of this monoclonal antibody in the long term treatment of patients with uveitis<sup>20</sup>. Patients who previously needed systemic immunosuppression to control their uveitis could be taken off of this standard therapy and maintained only with the antibody therapy given at monthly intervals. **This new therapy may permit long-term treatment of patients with severe uveitis with many fewer side effects than existing therapies, leading to an improved quality of life.** Further, these results have led others to adopt this treatment strategy with particularly promising results in multiple sclerosis patients. Planning is underway to begin a Phase III study to evaluate the full potential use of this therapy.







### CHEMOKINE MCP-1 ROLE IN UVEITIS

MCP-1 is a cellular protein that chemically attracts many inflammatory cells including monocytes, memory T-cells, natural killer cells, and mast cells. Scientists have examined the role played by MCP-1 in acute uveitis<sup>21</sup>. MCP-1 deficient mice and control mice were analyzed in a chemically induced rodent model of uveitis. The disease was markedly reduced in the MCP-1 deficient mice. Moreover, ocular inflammation was re-induced in MCP-1 deficient mice by administration of the MCP-1 protein. These data indicate that MCP-1 plays a crucial role in the induction of eye inflammation and may be a new therapeutic target for acute anterior uveitis.

### THE ROLE OF THE THYMUS IN UVEITIS

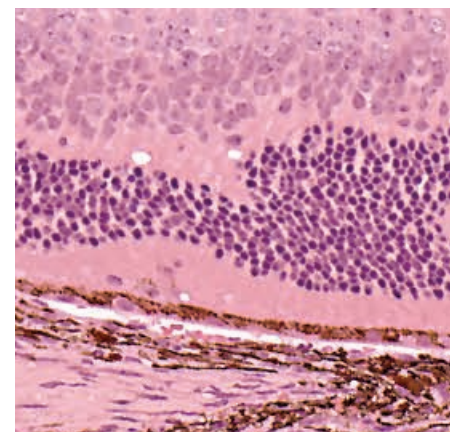
A collaborative team of researchers has found new evidence that the thymus gland plays a critical role in the development of uveitis. Located in the chest cavity, the thymus acts as a programming center for the immune system. Immature lymphocytes created from bone marrow stem cells enter the thymus and develop into a wide variety of T cells (T stands for thymus) that have affinity for infectious agents that invade the body. The thymus also contains a catalog of tissue-specific self-antigens. Self-antigens are those cell elements that hold potential to initiate an autoimmune response.

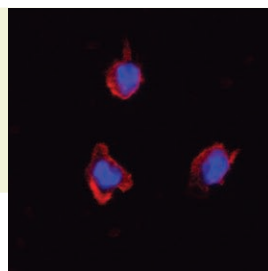
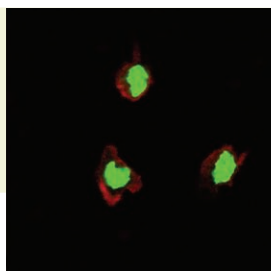
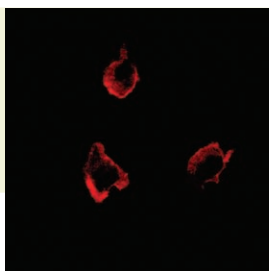
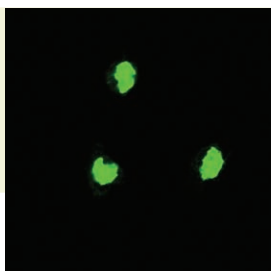
Developing T cells that have affinity for these self-antigens are eliminated in the thymus, thus creating tissue tolerance and preventing the potential for autoimmune diseases. In the current study, NEI scientists found that human thymus samples contained several eye-related self-antigens that have been previously associated with the development of uveitis in animal models<sup>22</sup>. However, the expression of these tissue antigens varied remarkably among the individual samples. Previous studies have found evidence that susceptibility to autoimmune disease decreases when the level of expression of a self-antigen within the thymus is robust. These findings greatly clarify the mechanisms that determine the susceptibility to uveitis. The discovery that the thymus plays a role in the disease also opens a new avenue of investigation to expand our understanding of uveitis and to develop therapies that prevent the disease or limit vision loss.

### HOST DEFENSE SYSTEM

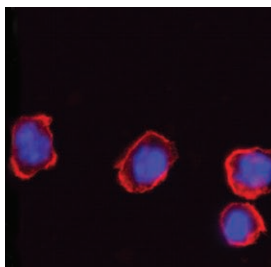
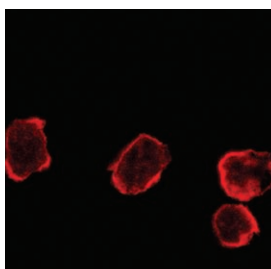
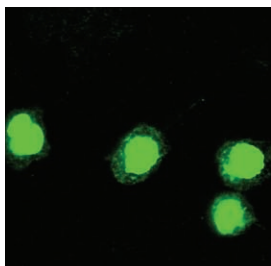
The body defends itself against bacteria and viruses in part by initiating an innate immune response that activates within minutes after the invasion of an attacking microorganism. Recent studies identified that in the innate immune response, the body recognizes foreign invaders through toll-like receptors (TLRs) that are present on the surface of selected cells within the body. When TLRs are engaged, genes important for an effective host defense, such as

those that cause inflammation, are activated. To investigate the role of TLRs in cells of the visual system, NEI intramural scientists analyzed TLR expression in human retinal pigment epithelial (RPE) cells<sup>23</sup>. The RPE cell layer provides a barrier between the body's blood supply and the neural retina and is thought to be a basic component of ocular immunity. Previous studies have found that RPE cells are a target of several infectious agents that can lead to vision loss. One particular TLR that defends against viral infection, TLR-3, was found to be highly expressed in these cells. Stimulation of TLR-3 receptors in RPE cells initiated an immune response that included the secretion of interferon beta, a molecule highly effective in inhibiting virus replication. This discovery identifies the central role of the TLR system and the immediate protection mechanisms of the innate immune response within the retina. Learning how the body quickly turns on genes and produces molecules that can protect the retina from invading organisms is crucial to designing augmented responses to viral infections of the retina.





ACROSS: T cells at rest  
DOWN: T cells stimulated



### AUTOIMMUNITY AND ALLERGY

Immune cells known as T helper type 1 (Th1) or type 2 (Th2) play important roles in defending the body against pathogens by producing different types of secreted proteins known as cytokines. Th1 cells protect against intracellular pathogens such as bacteria by secreting copious amounts of the cytokines interferon gamma ( $\text{IFN}\gamma$ ) and tumor necrosis factor alpha ( $\text{TNF}\alpha$ ). In contrast, Th2 cells, which are needed to control infections caused by extracellular parasites such as flatworms and roundworms, secrete specific cytokines known as interleukins (IL-4, IL-5, and IL-13). Cytokines function by instructing the cells of the body when to begin or stop growing, when to multiply, how long to live, and when to die. Although cytokines are important for many biochemical processes and are clearly beneficial in controlling diseases ranging from cancer to a variety of infectious diseases, their excessive production can be detrimental to normal cells. For example, persistent secretion of these cytokines is associated with allergies and autoimmune diseases such as uveitis, multiple sclerosis, rheumatoid arthritis, and type 1 diabetes.

Considerable evidence now indicates that the immune response of individuals that develop autoimmune diseases is dominated by Th1 cells that produce  $\text{IFN}\gamma$  while predisposition to allergic diseases is associated with elevated levels of Th2 cells and Th2 cytokines. A desired clinical outcome for these patients is to control the level of production of the toxic cytokines that cause autoimmune or allergic diseases. Given the importance of maintaining appropriate levels of cytokines in normal cells, it is therefore not surprising that cytokine activities are themselves under stringent control. Scientists have identified a new family of proteins called suppressors of cytokine signaling (SOCS) which are also produced by T cells.

These proteins are unique in that they regulate not only the intensity and duration of the activities of cytokines, but they also inhibit other related cytokines. With the aid of newly developed high sensitivity technology, investigators were able to show that Th1 and Th2 cells differ significantly in the SOCS proteins they produce<sup>24</sup>. The parent cell from which Th1 and Th2 cells derive produces relatively low levels of SOCS proteins. However, its differentiation into either Th1 or Th2 cells is accompanied by dramatic changes in the levels and types of SOCS proteins it produces. Those cells that are destined to become Th2 cells produce levels of SOCS3 that are 23 times higher than the amount made by Th1 cells. In contrast, Th1 cells produce much higher amounts of SOCS1 than their Th2 “siblings.” These results suggest that mutually exclusive patterns of cytokine expression by Th1 and Th2 cells may derive in part from SOCS3- or SOCS1-mediated repression of requisite signaling pathways in Th2 and Th1 cells.

SOCS1 and SOCS3 proteins are therefore T-cell lineage markers that can serve as therapeutic targets for immune modulation therapy. Drugs such as Remicade, Enbrel and Zenapax that inhibit cytokine activities are currently used to treat patients with uveitis, rheumatoid arthritis or other inflammatory disease. However, each of these drugs targets only a single cytokine. Because



inflammatory diseases are characterized by production of large amounts of a wide array of cytokines and SOCS proteins inhibit activities of multiple and diverse cytokines, therapeutic targeting of SOCS proteins would provide a more effective anti-inflammatory therapy.

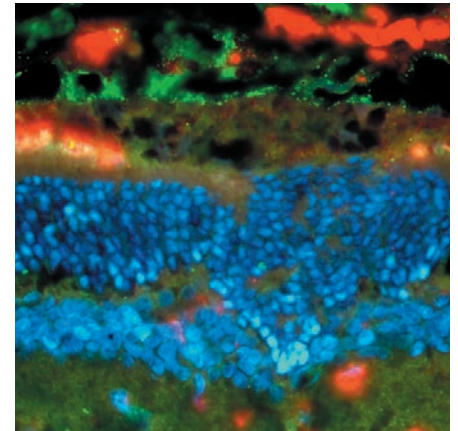
#### INHIBITION OF VIRAL REPLICATION

Cytomegalovirus (CMV) is a common herpes virus that usually causes sub-clinical or mild infection in adults with healthy immune systems. However, in AIDS patients or organ transplant recipients, whose immune systems are suppressed by the disease or by medication, the virus can spread throughout the body and cause serious complications. In the eye, the virus causes CMV retinitis, a condition that leads to vision loss. The inflammatory response to CMV retinitis can also cause a decrease in vision. There continues to be a need for innovative therapies and for model systems in which to test them.

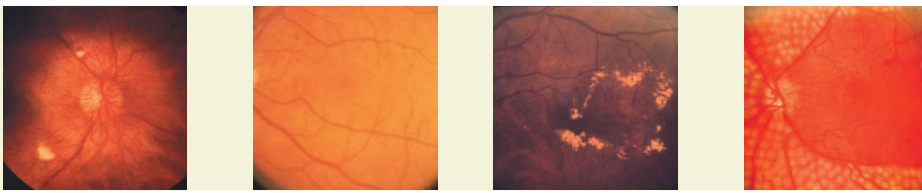
One such therapeutic approach involves antisense oligonucleotides, compounds that block the production of viral proteins within cells by interfering with the messages directing their production. Scientists used two model systems for growing cells in the laboratory for testing two oligonucleotides that have been developed against CMV<sup>25</sup>. One system employs human fibroblasts (skin cells) and the other human retinal pigment epithelial (HRPE) cells, derived from a layer of cells in close contact to retinal photoreceptors. The researchers found that both oligonucleotides were effective in inhibiting viral damage to the cells. Interestingly, they found the oligonucleotides were effective up to six days after introduction of the virus in HRPE system but only up to three days in the fibroblast system. This study demonstrates not only the antiviral activity of the oligonucleotides being studied, but also their increased potency in the HRPE system. The investigators suggest that this may be due to the slow spread of the virus through the HRPE cells compared to a more rapid spread in fibroblasts. Slow spread of the virus through the HRPE system more closely parallels the slow, progressive spread of the virus seen in patients with CMV retinitis. This may make the HRPE system a more appropriate system for testing other antiviral therapies.

#### DAMAGE TRIGGERED BY INFECTION

Ocular tissue damage is caused, in part, by the manner in which the host responds to an insult, such as an infection. The protozoan parasite, *Toxoplasma gondii*, which can cause toxoplasmosis, infects millions of people worldwide. This organism is also the most frequently identified cause of ocular inflammation, and the retinal damage is an initiator of blindness in young adults. Scientists at the NEI demonstrated in the laboratory that *T. gondii* infection of specific cells in the retina can cause secretion of molecules that contribute to the damage of ocular tissues<sup>26</sup>. These findings identify key molecules produced by retinal cells in response to infection and suggest therapeutic strategies to prevent retinal tissue damage that can be explored in future studies.







## Retinopathy of Prematurity

Retinopathy of Prematurity (ROP), a blinding eye disease common to premature, low birthweight infants, occurs when the gestational development of the retina is disrupted by premature birth. Lacking a complete vasculature, the retina is deprived of oxygen. The sudden, unexpected demand for oxygen spurs the proliferation of chaotic and poorly formed blood vessels that infiltrate the retina and vitreous—the clear jelly-like substance inside the eye. The faulty vasculature leaks serum and blood and forms scar tissue on the retina. In more severe stages of the disease, the scar tissue contracts, pulling on the delicate retinal tissue and causing retinal detachment. Each year ROP affects an estimated 14,000–16,000 premature, low birthweight infants in the United States, making it a leading cause of vision loss in children. It is hoped that progress that has been achieved in treating this disease will lessen its impact on these children.

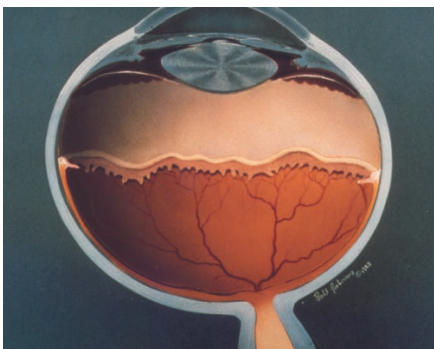
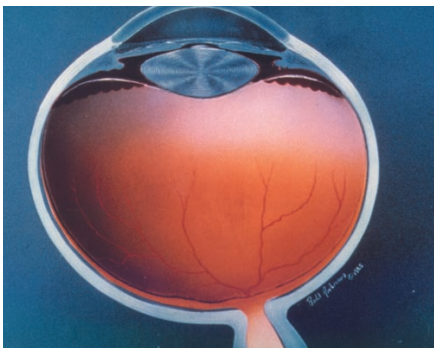
### EARLY TREATMENT

ROP follows an unpredictable course, presenting doctors with difficult treatment decisions. In most cases the vessels spontaneously regress, sparing vision. But less frequently the pathology continues, requiring laser therapy or cryotherapy to preserve the most critical component of sight: central vision. Clear central vision allows us to read, recognize faces, and perform a multitude of tasks that require hand-eye coordination. Although these therapies are beneficial in preserving central vision, they cause profound damage to peripheral vision. Owing to a lack of clinical criteria to predict which patients will require therapy, ophthalmologists have been forced to defer treatment until it was clearly indicated. Unfortunately, delaying therapy to avoid undue morbidity in the majority of patients leaves infants who most require treatment with poor visual outcomes.

Results from a National Eye Institute-sponsored clinical trial, called The Early Treatment of Retinopathy of Prematurity (ETROP), established that a computerized risk model was able to identify high-risk infants early in the disease<sup>27</sup>. The risk model assesses criteria associated with ROP such as birthweight, ethnicity, being a single or multiple birth baby, gestational age, and ophthalmic exam findings. The ETROP study also established that premature infants at highest risk of vision loss from ROP will retain better vision when therapy is administered in the early stage of the disease. **The results of the ETROP study provide the pediatric ophthalmology community with new tools to improve the quality of life for premature, low birthweight infants.**

### SUPPLEMENTAL OXYGEN

Many premature infants need supplemental oxygen soon after birth because their lungs are not sufficiently mature to efficiently transfer oxygen into their bodies. Researchers have long known that high levels of supplemental oxygen, while helping infants survive, might increase cases of ROP. In order to test the safety and efficacy of providing infants carefully-regulated supplemental oxygen, the NEI-supported the Supplemental Therapeutic Oxygen for Pre-threshold ROP (STOP-ROP) study<sup>28</sup>. Researchers found that supplemen-



TOP: Normal Eye  
BOTTOM: Eye with ROP



**SIMULATION**  
View seen with Diabetic Retinopathy  
(Normal Vision view on page 8)



*Advances in Retinal  
Disease Research*

tal oxygen given to premature infants with moderate ROP did not improve or worsen the condition. The results mean that clinicians can be less restrictive in administering supplemental oxygen to infants with moderate ROP.

## Diabetic Retinopathy

Diabetic retinopathy is a complication of diabetes and a leading cause of blindness. It occurs when diabetes damages blood vessels inside the retina, the light-sensitive tissue at the back of the eye. In the early stages of the disease, blood vessels become swollen and blocked. This can cause macular edema, a build up of fluid on the central portion of the retina. Patients with macular edema first experience blurred vision that can lead to permanent vision loss. Eventually, as more and more of the retinal vasculature becomes blocked, it can no longer keep up with the retina's demand for oxygen and nutrients. This spurs the growth of abnormal, fragile blood vessels that leak blood into the retina, causing a profound loss of vision. Between 40 to 45 percent of Americans diagnosed with type 1 or type 2 diabetes have diabetic retinopathy, making it a leading cause of vision loss in working-age adults. The NEI has supported research to reduce vision loss from diabetic retinopathy and improve treatments for more than 30 years, and current therapies are now highly effective in reducing the risk of vision loss due to diabetes.

### DIABETIC RETINOPATHY TREATMENT

Treatment strategies for diabetic retinopathy developed over the last several decades are, in part, based on NEI-supported clinical trials of laser photocoagulation, and vitrectomy, a surgical procedure where the clear, jelly-like fluid in the eye is removed. These treatments were found to be 90 percent successful in reducing the risk of severe vision loss over short term, however, the long term effects of these treatment strategies were not known. A follow-up study of Early Treatment Diabetic Retinopathy Study patients from one treatment center was conducted at the National Eye Institute, 13 to 19.5 years after the initial laser photocoagulation (median, 16.7 years)<sup>29</sup>. Although the mortality rate of patients with diabetic retinopathy is much higher than that of the general population, clinician scientists found that aggressive follow-up, with treatment when indicated, is associated with maintenance of good long-term visual acuity for most patients. Vigilant follow-up is essential in maintaining good vision in patients with diabetic retinopathy. This underscores the need to increase screening for diabetic retinopathy and the delivery of timely treatment in patients with diabetes.

### DIABETIC RETINOPATHY IN THE MEXICAN-AMERICAN POPULATION

Mexican-Americans are known to have a high rate of diabetes along with more severe hyperglycemia, which indicates poor glucose control. This can lead to major complications of diabetes, including severe diabetic retinopathy and blindness. As a means of determining the causes and prevalence of blindness and visual impairment in the U.S. Mexican-American population, the NEI-supported the study Proyecto VER (Vision Evaluation and Research)<sup>30</sup>. This study assessed visual impairment in a population-based sample of 4500 Mexican-Americans age 40 or older, residing in Tucson and Nogales, Arizona. The rate of diabetes in this group was 20 percent, twice the rate reported for non-Hispanic Whites. The rate of diabetic retinopathy in those with diabetes was 48 per cent, a rate similar to that of non-Hispanic Whites. Importantly, 15 percent of study participants did not realize they had diabetes and were newly diagnosed by the study. Among this group of newly diagnosed diabetics, 23 percent had early to moderate diabetic retinopathy, a potentially blinding eye complication of diabetes. Another nine percent had advanced diabetic retinopathy and were in immediate danger of losing some vision. This study points to the importance of early diagnosis and treatment of diabetes and the importance of regular, dilated eye exams in this high-risk, minority population and underscores the need for the NEI's National Eye Health Education Program to continue targeted health education activities and messages directed to addressing these concerns.



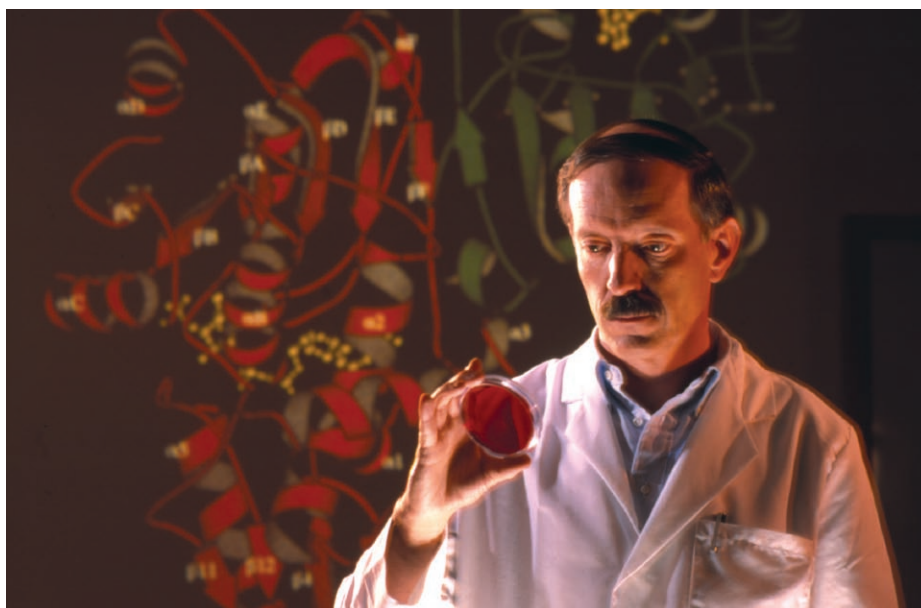


## Ocular Melanoma

Choroidal melanoma is the most common primary eye cancer in adults. Removal of the eye, known clinically as enucleation, has been the standard treatment for choroidal melanoma for over a century, because the melanoma cells also can spread to other parts of the body and cause death. Radiation therapy emerged twenty years ago as a method to possibly preserve vision and reduce mortality. Research over the past few years has been instrumental in determining the factors that improve prognosis subsequent to treatment.

### COLLABORATIVE OCULAR MELANOMA TRIAL

The Collaborative Ocular Melanoma Study (COMS) was supported by the NEI and NCI to compare enucleation versus radiation with respect to survival<sup>31</sup>. Investigators previously reported that patients with tumors large enough to require removal of the eye, who were randomized either to receive radiation treatment to the affected eye before it was removed or to having the eye removed without radiation treatment, showed similar five-year survival rates of 60 percent. These researchers have recently reported that patients with medium-sized tumors, who were randomized either to receive radiation therapy or to have the eye removed, also had similar five-year survival rates of 82 percent<sup>32</sup>. These results reveal that the size of the tumor is the most critical factor in influencing prognosis and emphasize the importance of early detection and treatment. With the data also showing similar survival rates for radiation therapy versus removal of the eye, quality of life issues become important factors in helping the patient and doctor determine treatment options.





## Genetics and Gene Therapy

Significant progress has been made over the past several years in cataloguing and mapping genes expressed in the visual system. Over 100 genes have been linked to specific retinal diseases. As the field of genomics and associated technologies has expanded, important new opportunities have arisen in understanding the relation of specific genes and gene variations or mutations to the disease process so that ultimately new therapeutic strategies can be developed.

### MITOCHONDRIAL GENE THERAPY

Leber's Hereditary Optic Neuropathy (LHON) is an inherited disease characterized by sudden visual failure in individuals between 20 and 30 years of age. Half of these affected individuals will become totally blind. Ninety-five percent of these cases can be linked to three different DNA mutations in mitochondrial DNA rather than in the nuclear DNA that codes for most proteins. Mitochondrial DNA codes for 13 proteins that are essential for the energy production required for metabolic processes in living organisms. The repair of mitochondrial DNA is a special challenge, because it is not possible to directly incorporate genes into mitochondria. Researchers supported by the NEI were able to overcome this roadblock by introducing the corrected mitochondrial gene into the nucleus of the cell<sup>33,34,35</sup>. The corrected gene contained a highly novel targeting sequence that incorporated the gene's protein product into the mitochondria. The protein restored normal metabolic function. Future research will focus on understanding the pathophysiology of LHON to provide more precise tools for a gene-based treatment of this disorder in patients.

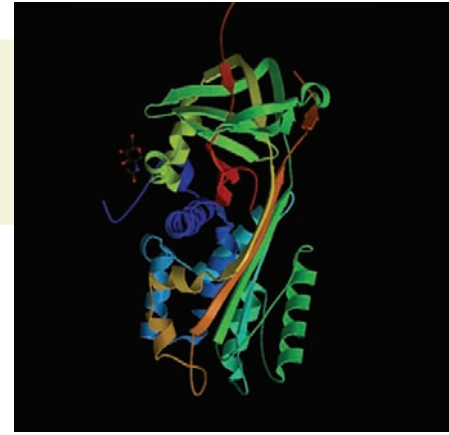
### RNA INTERFERENCE GENE THERAPY

NEI-supported researchers are exploring the use of a new gene therapy approach, called RNA interference, in treating eye disease. In nature, organisms are equipped with genetic sequences that regulate gene expression to insure that a cell encodes the required quantities of a protein needed to function and survive. If a gene produces too much of a protein, it can have a detrimental affect. RNA interference is a naturally occurring biological process that cells employ to control the expression of genes. When a gene initiates the production of a protein, it releases a copy of the coding sequence from the nucleus of the cell. The cell then reads the sequence, called messenger RNA (ribonucleic acid), to construct a protein. RNA interference causes destruction of the messenger RNA, thus preventing the production of the protein. Borrowing from nature, NEI-supported researchers have recently developed synthetic RNA interference sequences to prevent the expression of a gene for vascular endothelial growth factor (VEGF)<sup>36</sup>. As its name implies, VEGF spurs blood vessel growth. NEI-supported researchers have previously found increased protein levels of VEGF in patients with macular degeneration and diabetic retinopathy. In animal models of disease, RNA interference blocked VEGF gene expression

and prevented the growth of blood vessels. Continuation of this line of research not only has implications for treatment of a variety of diseases, but it will also help researchers to gain insight into a gene's function in health and disease and study its role in development.

### GENE THERAPY FOR DOMINANTLY- INHERITED RETINAL DISEASES

Retinal diseases offer promising targets for gene therapy, since over 100 different genes are known to be involved in causing retinal disorders. In dominantly inherited diseases, only one of the pair of corresponding genes normally found on a chromosome need be mutated to cause the disease. Ribozyme gene therapy has the potential to overcome dominant diseases by deactivating the mutated gene and leaving the corresponding normal gene partner intact and functional. Normally during gene expression, a messenger RNA (mRNA) molecule is produced that is read within the cell to direct the assembly of a gene product. Ribozymes are small RNA molecules that target and then splice mutant mRNA at the mutation site, thus deactivating the defective mRNA but leaving the mRNA from the normal gene intact. Recent experiments in animals have demonstrated that it is possible to deliver ribozymes to photoreceptor cells and decrease the number of mutant mRNA molecules made from a known dominant mutation in the rhodopsin gene that causes a common form of RP<sup>37</sup>. This treatment effectively cured the retinal degeneration for up to eight months. Research will continue into the application of this therapy to other dominantly-inherited, single-gene mutations that cause retinal degenerative diseases.



### NEUROTROPHIC GENE THERAPY

NEI-funded researchers have found that a family of naturally occurring proteins in the body maintains the health of retinal cells. These proteins, known collectively as neurotrophic agents, have a wide degree of effectiveness in many different rodent models of retinal degenerative disease, making them desirable candidates for therapy. However, these proteins cannot cross the blood/retina barrier, thus preventing their administration by traditional systemic delivery methods such as injections. Neurotrophic gene therapy provides a novel way to overcome this obstacle by delivering the genes that encode these proteins. **Using neurotrophic gene therapy with the ciliary neurotrophic factor (CNTF) gene, NEI-supported researchers have preserved photoreceptor cells in rodent models of retinitis pigmentosa<sup>38</sup>.** Recent NEI-supported laboratory studies have also found that neurotrophic gene therapy with brain-derived neurotrophic factor (BDNF) preserves retinal ganglion cells in a rodent model of glaucoma<sup>39</sup>. In a novel spin on neurotrophic gene therapy, Neurotech, a small biotechnology company based in Rhode Island and France, has transfected retinal cells with the CNTF gene and encapsulated them in an implantable device that allows CNTF to diffuse into the retina<sup>40</sup>. The device, called Encapsulated Cell Technology (ECT), is now being evaluated in clinical trials being conducted at the NEI.

### REGULATING GENE EXPRESSION

One of the major concerns surrounding gene therapy is the need to regulate gene expression. Too high a level of a therapeutic protein can have detrimental effects and too

little protein will fail to achieve a therapeutic outcome. Also, the ability to shut off gene expression is a critical safety concern in clinical trials. To address these concerns, NEI-supported investigators have created inducible promoters to regulate the amount of protein produced by a therapeutic gene. One such inducible promoter contains regulatory sequences that activate or deactivate gene expression when exposed to tetracycline, a commonly used antibiotic<sup>41</sup>. This system can be used to turn gene expression on or off and increase expression by oral administration of very small doses of the drug. Such a system would allow for safe and long-term administration of gene therapy.

### ANTI-NEOVASCULAR GENE THERAPY

As humans develop and grow, a host of genes are expressed to form new blood vessels. This process, called neovascularization, stops at adulthood. However, eye diseases like macular degeneration and diabetic retinopathy cause some of the genes associated with neovascularization to reactivate, spurring the development of poorly formed, abnormal blood vessels in the back of the eye. These vessels leak fluid and blood into the macula—the central portion of the retina that helps us perceive colors and fine visual detail—causing severe vision loss. Researchers at the NEI discovered a gene called pigment epithelial-derived factor (PEDF) that was later found to prevent neovascularization in animal models that mimic macular degeneration.<sup>42</sup> Further NEI-supported research found that anti-neovascular

gene therapy with PEDF not only prevents neovascularization but also causes newly formed blood vessels to regress<sup>43</sup>. The safety of this anti-neovascular gene therapy treatment is now being evaluated in patients with macular degeneration in a phase one clinical trial sponsored by GenVec.

### RODENT MODEL OF RETINITIS PIGMENTOSA

Named for the British medical institution where it was discovered in 1938, the Royal College of Surgeons (RCS) rat is the most widely studied animal model of retinal degeneration. The RCS rat has been used extensively to evaluate experimental therapies for RP. Despite its wide-spread use, the genetic cause of the degeneration has remained a mystery. Recently, however, NEI-supported investigators discovered that the RCS rat has a mutation in the *Mertk* gene<sup>44</sup>.

The *Mertk* gene and its protein product are expressed in a layer of cells that adjoin photoreceptor cells called the retinal pigment epithelium (RPE). RPE cells perform a number of tasks that are critical to the health and function of photoreceptor cells. For example, RPE cells convert vitamin A into a chemical derivative (11-cis-retinal) that is necessary for vision. RPE cells shuttle oxygen and nutrients from the blood supply to photoreceptor cells. RPE cells also digest and recycle waste products created by photoreceptor cells.

One major source of waste products is the outer segment tips that photoreceptor cells shed during





sleep. Outer segments, which under the microscope resemble a roll of wrapped pennies, absorb light and turn it into an electrical signal that is eventually relayed to the brain. In a process called phagocytosis, RPE cells digest these spent outer segment tips and recycle their component vitamin A byproducts and fats back to the photoreceptor cells. The photoreceptor cell then uses these recycled materials to replenish its outer segments. This process results in a complete renewal of an outer segment in about ten days.

The *Mertk* gene is thought to play an important role in this continual renewal of outer segments. Researchers have known for some time that in the RCS rat, RPE cells are unable to ingest outer segments. The shed disks accumulate between the RPE and photoreceptor cell layers and, starting at the age of 21 days, the RCS rat experiences a rapid degeneration of photoreceptor cells. More recent investigation suggests that the *Mertk* gene encodes a protein that in some way allows the RPE cell to ingest the spent outer segment tips from the photoreceptor cells. Mutations in the *Mertk* gene prevent the RPE cell from ingesting outer segment tips and recycling their renewable resources. This accumulation of outer segments leads to rapid vision loss.

Mutations in the *Mertk* gene were subsequently found in humans with severe RP. Although this form of RP is aggressive, years of studying the RCS rat have led to several therapeutic breakthroughs that might have application to treating the disease in humans. Gene therapy, transplantation and drug therapies have all successfully prevented vision loss in this model.

Discovery of the *Mertk* gene in the RCS rat and humans now make it possible to contemplate translational approaches to treating the disease.

#### **GENE FOR HALLERVORDEN-SPATZ SYNDROME**

Hallervorden-Spatz syndrome (HSS) is a rare, inherited, neurological disorder associated with high accumulations of iron in the brain, and causes progressive degeneration of the retina and nervous system. In addition to a number of neurological symptoms that develop during childhood, some patients also develop degeneration of the retina. Death usually occurs in early adulthood, approximately 10 years after onset. Scientists recently discovered a defective gene that produces an ineffective enzyme in patients with HSS<sup>45</sup>. The enzyme is needed by the body to use vitamin B5, which is required to produce some of the body's essential compounds. These researchers hypothesize that the production of the ineffective enzyme by the defective gene causes blockage of normal metabolism and the accumulation of metabolic materials resulting from that blockage. It is believed that this accumulation results in degeneration of the retina and high concentration of iron in the neural tissues. Research is now being focused on developing treatment strategies that bypass this defective enzyme, allowing the body to use vitamin B5 to help make essential components. Understanding the biochemical defects in HSS may also provide insights into the effect iron has on other neurodegenerative diseases associated with high iron accumulations, such as Parkinson's disease.

#### **RPE65 AND LRAT IN THE VISUAL CYCLE**

Vitamin A and its derivatives are critical components to vision. In photoreceptor cells, a vitamin A derivative, called 11-cis-retinaldehyde, combines with a protein called opsin to form rhodopsin. Rhodopsin is the key molecule that absorbs light and begins the cascade of molecular events that converts light to the chemical and electrical signals that our brains process to visualize our surroundings. When light hits rhodopsin, 11-cis-retinaldehyde is changed to another vitamin A derivative called all-trans-retinaldehyde. However, once rhodopsin is converted to this form, it can no longer absorb light. Through a process called the visual cycle, 11-cis-retinaldehyde is renewed so that it can again participate in the visual process. Studying the gene expression underlying the visual cycle, scientists discovered that a previously known protein called RPE65 is responsible for the chemical conversion of spent rhodopsin<sup>46</sup>. Mutations in the RPE65 gene are known to cause a range of retinal degenerative diseases that vary widely in severity. For example, some mutations are associated with Leber's congenital amaurosis, an eye disease that causes blindness in infants, while others result in mild to moderate forms of retinitis pigmentosa. A more precise understanding of RPE65 will help clarify our knowledge of the visual cycle and the diverse diseases that emerge from alterations in this gene.

Building on the previous study described above, scientists used an artificial visual cycle developed in cell culture to study RPE65 function. Importantly, they found that although RPE65 is the only protein

product needed for renewal of 11-cis-retinaldehyde, robust production of this vitamin A derivative relies on an enzyme called lecithin retinol acyltransferase (LRAT)<sup>47</sup>. Previous studies have found that mutations in the gene that encodes LRAT also cause a severe, early onset retinal degenerative disease. LRAT was found to provide the immediate precursor to 11-cis vitamin A and induce RPE65 activity. Results from the cell culture study also revealed that an iron atom was required in the renewal process, establishing the previously unappreciated role of iron in the visual cycle. In total, these two studies confirm that RPE65 is essential to the visual cycle and that LRAT acts in concert with this protein. **Gene therapy trials for blindness due to mutations in RPE65 have been successful in restoring vision in mice and dogs lacking RPE65 and are now planned for humans.** The new results confirm that RPE65 gene therapy directly targets the key component of the defective process causing this form of blindness.

#### **GENETIC BASIS OF STARGARDT MACULAR DYSTROPHY**

Stargardt macular dystrophy is associated with an inherited progressive loss in central vision. Affected individuals have progressive degeneration of the fovea (a specialized region of the central retina or macula that is rich in a type of cone photoreceptor cells). This disease is associated with mutations in the ABCA4 gene that encodes a protein which is found in cone and rod photoreceptor cells. NEI-supported scientists have found that ABCA4

protein facilitates the removal of chemical by-products that result from a photoreceptor's response to light from the neural retina.<sup>48</sup> In Stargardt disease, these by-products accumulate in the retina and lead to the degeneration and death of cone cells in the macula. Further studies may suggest ways to prevent or treat this disease more effectively.

#### **MOUSE MODEL FOR MACULAR DEGENERATION**

Stargardt-like macular dystrophy (STGD3) is a rare, inherited form of juvenile macular degeneration that shares many clinical features with AMD, including the accumulation of deposits called lipofuscin in the retina prior to the degeneration of the photoreceptor cells. As of yet, there are no suitable animal models for AMD, and so the development of a rodent model for STGD3 may offer insights into both diseases. In 2001, alterations in a gene called ELOVL4 were found to cause STGD3. This year, vision researchers developed a transgenic mouse model with a mutant form of the ELOVL4 gene<sup>49</sup>. The mutant ELOVL4 mice were found to possess some of the hallmarks of macular degeneration, including accumulation of lipofuscin in the retina, abnormal neural activity and localized degeneration of photoreceptor cells in the retina. The availability of the ELOVL4-mutant mouse will facilitate our understanding of the basic pathogenesis of macular degeneration and offer a model to evaluate therapeutic interventions.

#### **GENE THERAPY FOR UVEITIS**

Researchers at the National Eye Institute in collaboration with scientists from the American Red Cross devised a gene therapy approach to reinstate functional tolerance to a retinal antigen and tested it in a mouse model of uveitis<sup>50</sup>. A single infusion of this gene therapy was able to protect mice from developing uveitis. Importantly, it also reversed uveitis in mice with active disease. This treatment approach could have wide spread application for other autoimmune diseases such as multiple sclerosis.

#### **VISUAL SYSTEM GENES**

An important barrier to therapeutic intervention in human retinal disease is the identification of the gene or genes that cause vision loss. Vision loss resulting from degenerative and other changes in the retina are largely linked to rod and cone photoreceptors. Scientists have recently undertaken a comprehensive genetic analysis of rod photoreceptors, the most abundant sensory neuron in the retina, in order to identify all the possible genes expressed in these cells<sup>51</sup>. Rod cells play an essential role in the visual pathway and may be especially vulnerable to any genetic defect involving the retina. A complete database of rod-specific genes would simplify the identification of candidate retinal disease genes. Using the technique of serial analysis of gene expression, or SAGE, in both mature and developing retinal tissues, many new genes in rod photoreceptors have been identified. This work represents an important new strategy for the study of retinal disease.





## Retinal Neuroscience

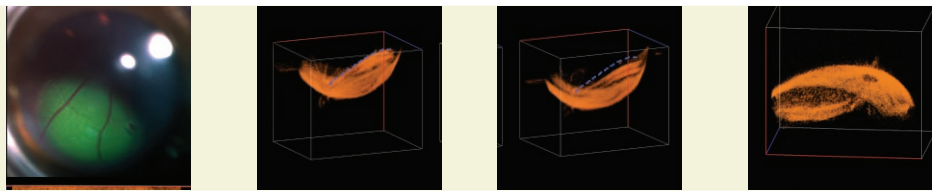
Vision is the end product of a complex neurologic system. To understand how disease and injury adversely affect vision, we must fully understand how the various cells and tissues interact in this complex neural network to permit the formation of images and the discrimination of colors. Our understanding of the roles, interactions, and relationships of the neurons within and that lead from the retina has increased over the past few years, as has our knowledge of the genes that control their development and function.

### BIOLOGICAL CLOCK

The circadian (daily) clock synchronizes the biological activities of an organism to environmental changes such as light and darkness, and temperature. Within this 24 hour cycle or rhythm there are variations in mental alertness, sleep-wake patterns, eating habits, and hormonal levels. Although scientists have known for some time that this circadian clock is controlled or reset by light, the cellular events that utilize light to synchronize the circadian clock have remained a mystery until recently. Vision requires light stimulation of specialized sensory neurons called photoreceptor cells in the retina. Other nerve cells in the retina known as retinal ganglion cells (RGCs) further encode this visual information, transmitting it via the optic nerve to higher visual centers of the brain, including a non-visual area, the suprachiasmatic nucleus (SCN). The SCN is the circadian rhythm pacemaker of the brain, driving daily biological activities. Damage to the SCN can lead to complete disruption of circadian-linked behaviors; however, the SCN can function even in the absence of retinal photoreceptors. Two recent research findings have helped understand how this can be so<sup>52,53</sup>. Scientists have described a protein, melanopsin, that is present in a subset of RGCs in the retina. Melanopsin belongs to a family of proteins, called photopigments or opsins that are found in retinal photoreceptors and are essential for vision. Those RGCs that contain melanopsin project to the SCN. Other researchers have shown that the RGCs projecting to the SCN respond directly to light stimulation. These findings indicate that those RGCs directly control the circadian pacemaker to drive cyclical biological activities. Future research in this area should provide a new framework to understand and ultimately control complex behaviors such as eating and awake activity.

### LIGHT ADAPTATION

Remarkably, the retina has the ability to adapt to changes in light intensity that allows us to see over a very wide range of illumination. The process by which the visual system changes its sensitivity, depending on the ambient light level, is called adaptation. The photoreceptor cells in the retina actually change their sensitivity. This is a slow process that may take many minutes until the visual system is fully adjusted to new light levels. NEI-supported scientists have reported a new cellular mechanism of rod photoreceptor adaptation that is triggered by daylight levels of illumination<sup>54</sup>. The mechanism involves a massive light-dependent translocation of the photoreceptor-specific protein, transducin, between functional compartments of a rod photoreceptor cell. The reduction in the transducin content of the rod photoreceptor outer segment was correspondingly accompanied by a reduction in rod cell activity. The finding that transducin physically translocates in response to light is most significant, not only for the process of light adaptation, but possibly more widely for the process of phototransduction. Previously light adaptation and light transduction were thought to involve only processes of saturation of the involved proteins by light. This new finding shows that this is not the case for adaptation, and suggests protein translocation should be considered for other steps in the process of signal transduction by photoreceptors.



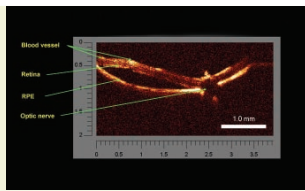
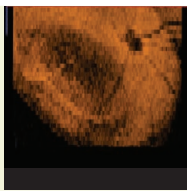
### VISUAL PROTEINS

Vision begins when light causes a change in the three-dimensional structure of rhodopsin, which initiates a cascade of events that result in vision. Understanding how the dynamic structure of rhodopsin acts as a trigger in the cascade of events leading to vision has been an important and difficult goal of scientists studying vision and vision loss. NEI-supported researchers have now succeeded in identifying, for the first time and at very high resolution, the three-dimensional structure of rhodopsin<sup>55</sup>. Mutations in the rhodopsin gene are related to many of the cases of retinitis pigmentosa, a group of inherited, blinding, retinal degenerations. This finding is significant because it will help scientists understand how structural changes in rhodopsin initiate the next step in the vision cascade, the activation of a protein known as G-protein. Elucidating the coupling of the rhodopsin receptor with the G-protein may provide a significant new avenue for the development of therapeutics to combat vision loss.

High resolution identification of the three-dimensional structure of rhodopsin also has broader implications for other biological systems besides vision. Rhodopsin is a member of the large class of G-protein-coupled receptors. These receptors respond to light or other stimuli such as hormones, calcium ion levels, or odorants to initiate a biological response. Continuation of this line of research will help explain how these receptors transmit signals and will have important implications for research in many biological systems.

### FUNCTION OF OSTEONECTIN

Osteonectin is a protein that was initially found in bone and that has been shown to be secreted by tissues throughout the body. Osteonectin is involved in cell growth, proliferation, and differentiation, and has anti-adhesive properties. Osteonectin has also been implicated in ocular disease. Osteonectin expression is increased in human age-related cataracts and a mouse model that is deficient in osteonectin develops an age-related cataract. NIH intramural scientists have also been studying the function of this protein in the retina<sup>56</sup>. The macula is the portion of the retina that provides sharp central vision. Osteonectin mRNA is expressed in 8-10 fold greater levels in the macular retinal pigment epithelium (RPE)—the tissue that supports many of the retina's metabolic functions—than in the peripheral RPE. The osteonectin protein has been detected in the macula and in the peripheral neural retina, but only traces are found in the peripheral RPE and choroid. Immunocytochemical studies have localized osteonectin mostly in the outer plexiform layer (OPL)—the part of the retina containing the axons of the photoreceptor cells—but also in the macular RPE. These data suggest osteonectin is synthesized by the macular RPE and is then secreted and transported to the OPL. The increased levels of the mRNA in the macular RPE may be due to greater turnover of osteonectin in the macula versus the peripheral retina. The function of osteonectin in the retina is unknown, but its high levels of expression by the macular RPE and its localization to the OPL are unique and suggest that it may have an important role in macular function. Additional studies are in progress to further localize the sources of osteonectin in the retina as well as the structure(s) that it interacts with in the OPL.



## Technical Advances

Progress has also been made in technological capabilities that have important implications for patients and clinical and laboratory scientists. Research has continued toward the development of a means to restore visual function to those who have retinal damage due to disease or injury. Advances in imaging technologies now offer the potential to view the effect of a genetic mutation on protein structures within the retina or to detect the earliest pathologic changes that occur in a disease.

### THE RETINAL PROSTHESIS

The marriage of computer technology and medical science is creating advances in overcoming even the most intractable diseases. In one such union, specially designed computer chips implanted in the eye may make it possible to restore some measure of visual function to the blind. Retinal degenerative diseases such as retinitis pigmentosa damage and destroy the light-sensitive photoreceptor cells in the retina. Although these cells die, much of the remaining nerve cell network in the retina remains healthy. The microelectronic retinal prosthesis, a device developed by researchers at the University of Southern California, mimics the function of photoreceptor cells by turning light into electric signals. The device consists of an implantable computer chip that receives signals from a camera mounted on a pair of glasses. In a recently published study, a 74 year-old patient, who has been blind for more than 50 years from retinitis pigmentosa, was implanted with a retinal prosthesis<sup>57</sup>. The patient

then underwent 10 weeks of visual assessment. Using the device, the patient was able to see spots of light, detect motion, and recognize simple shapes. When the prosthesis was turned off, the patient could not perform any of these tasks. **Although preliminary, these results are a promising first step in realizing a prosthetic device that can restore ambulatory vision to patients with severe visual impairments due to retinal degenerative diseases, a major cause of vision loss in this country.**

### ATOMIC FORCE MICROSCOPY

Rhodopsin is a membrane protein in retinal photoreceptor cells that absorbs light and triggers a cascade of biochemical reactions which allow us to see. Many blinding visual disorders involve mutations in rhodopsin, or affect the structural integrity of the outer segment of the photoreceptor cells where rhodopsin is located. Knowing the structure and arrangement of the rhodopsin molecule in the photoreceptor outer segment membrane could provide a better understanding of the biochemical process underlying vision and provide clues to the disease mechanisms that cause retinal degeneration. The atomic

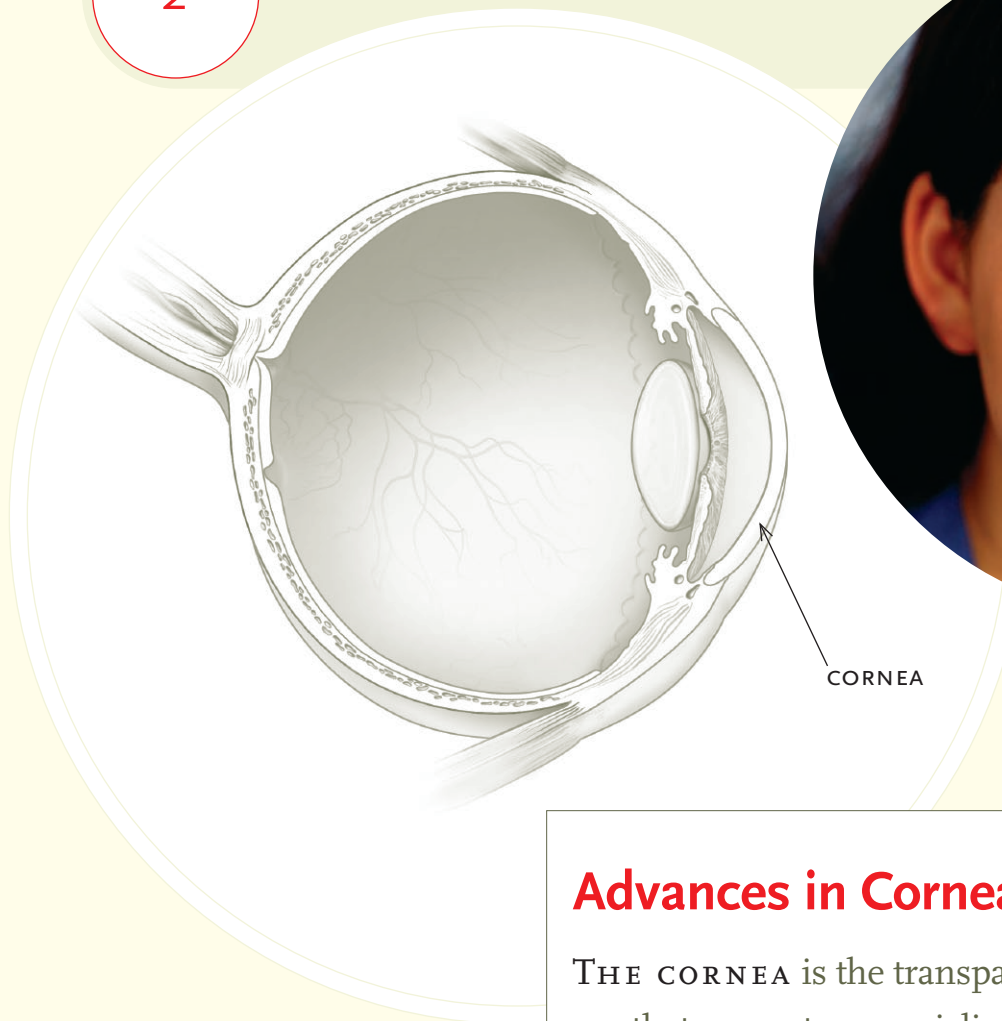
force microscope (AFM) is a new imaging technology that probes the surface of a sample with such a discriminating touch that it can even sense individual molecules in a biological preparation. Using AFM on photoreceptors, scientists created stunning AFM pictures which captured neat rows of paired rhodopsin molecules in their natural state<sup>58</sup>. The rhodopsin dimers (doublets) were very closely packed in paracrystalline arrays, increasing the sensitivity of the molecule to light. Future studies of photoreceptor cells with rhodopsin mutations may reveal whether rhodopsin is less tightly packed in these cases, affecting sensitivity, and whether other mutations in other parts of the visual cycle affect rhodopsin packing and therefore sensitivity.



### **OPTICAL COHERENCE TOMOGRAPHY**

Optical coherence tomography (OCT) is a non-invasive imaging technique, similar to ultrasound, that promises to have a broad range of applications for the diagnosis and management of a variety of ocular diseases. Currently available OCT instruments have a resolution of 10 micrometers. A prototype has been developed that can resolve retinal structures to a 3 micrometer level<sup>59</sup>. Results from these instruments demonstrate their ability to image retinal structures associated with a specific disease non-invasively, providing earlier diagnosis and more precise monitoring of progression. For example, thinning of the retinal nerve fiber layer is an indicator of glaucoma and its progression. Ultra-high resolution OCT can potentially detect changes in thickness in this layer down to the single cell level, thus providing greater sensitivity and precision than other ophthalmic imaging instruments. Ultrahigh resolution ophthalmologic OCT allows more precise examination of retinal morphology for earlier diagnosis and more precise monitoring of ocular pathologies. Practically, this may enable clinicians to intervene before extensive sight is lost and to monitor treatments more effectively. Other uses for this instrument include the diagnosis and management of diabetic retinopathy and macular degeneration. Glaucoma, diabetic retinopathy, and macular degeneration disproportionately affect people over 65. Early detection of these blinding diseases is critical in an aging population.





## Advances in Corneal Disease Research

THE CORNEA is the transparent tissue at the front of the eye that serves two specialized functions. First, it forms a protective physical barrier that shields the eye from the external environment. Second, it also serves as the main refractive element of the eye, directing incoming light onto the lens. Refraction depends on the cornea acquiring transparency during development and maintaining this transparency throughout adult life.



## Cornea Infections and Immune Processes

The cornea is susceptible to damage by injury owing to exposure to the external environment or by disease due to a variety of viruses, bacteria, fungi or other parasitic organisms. Because of the immune privilege of the eye, the corneal response to infections and injury is limited. Vision scientists have worked to increase our understanding of infections and immune responses that affect the cornea so that improved treatments can be developed.



### OCULAR HERPES SIMPLEX VIRUS INFECTION

Ocular Herpes simplex virus (HSV) infection is the most frequent serious viral eye infection and the leading cause of viral induced corneal blindness in the United States. The virus persists indefinitely in infected individuals. Up to 90 percent of adults possess circulating antibodies against the virus. After initial infection of mucous membranes in the mouth or the cornea of the eye, the virus invades the nerves and travels to sensory nerve cells that innervate the cornea where it may enter a dormant (latent) state that persists for the life of the infected individual. Reactivation of the latent virus occurs periodically and leads to reappearance and potential spread of virus on the surface of the eye. Repeated cycles of latency and reactivation can lead to progressive scarring and clouding of the cornea that, in turn, leads to blindness. Corneal infection is accompanied by periocular disease (spread of virus to the eyelids and conjunctiva) in over 50 percent of the acute infections. In order to determine whether these tissues are infected directly during the initial (primary) corneal infection, or are infected by newly formed virus in the nervous system, NEI-sponsored scientists employed new molecular genetic techniques to monitor and quantify the ap-

pearance of viral gene products in an animal model of HSV ocular infection<sup>60</sup>. This permitted tracking the time course of infection in cornea, the nervous system (trigeminal ganglia where the latent virus resides), and the surrounding ocular (periocular) tissues. The time course suggested that after HSV infects the cornea, the virus travels to the nerve cells in the trigeminal ganglia and after residing and quickly replicating in the nervous system, to the periocular tissues. Similarly, human spread of virus has a time course that closely mimics that observed in the animal model. This research suggests that in future treatment studies of acute, primary infection of the cornea, topical antivirals may be insufficient to limit disease, and rapid systemic treatment may be the best means of preventing spread of infection to these tissues.

### HERPES LATENCY

HSV causes a variety of diseases, including herpetic stomal keratitis in the cornea, as well as genital, brain, and skin afflictions. Subsequent to a primary infection, HSV establishes lifelong latent infection in nerve ganglia, followed by episodes of periodic reactivation. The long accepted explanation is that HSV persistence is due to the virus' ability to exist during latent periods without encoding any antigenic compo-



nents. It has been assumed that this absence of antigen expression allows the virus to escape recognition by the host immune system. However, recent work has found that immune T cells in latently infected ganglia are specific for HSV and are critical for regulation of HSV latency by inhibiting viral reactivation<sup>61</sup>. This research indicated that there is a dynamic balance between HSV-1 latency and reactivation involving a tripartite interaction among the virus, the host neuron, and local immune components. The findings of the host immune components in HSV latency should lead future HSV research in a new direction that will have a high impact in not only eye infection but a wide spectrum of human herpetic diseases.

#### **HERPES SIMPLEX VIRUS LATENCY AND MOVEMENT**

While survival of HSV in the dormant or latent state obviously requires survival of the host cells, infected cells normally undergo a process called programmed cell death, or apoptosis. Researchers recently discovered that a viral gene expressed during latency, the LAT gene, inhibits programmed cell death in infected nerve cells, thereby providing a host for the latent state<sup>62</sup>.

Other NEI-supported scientists have developed a model of HSV movement in nerves by injecting a known quantity of virus directly into a cluster of nerve cell bodies that allowed researchers to follow the movements of virus during reactivation and spread of the virus<sup>63</sup>. Movement of virus from the nerves to the corneal epithelial cell layers has a distinct pattern: the virus leaves the nerve endings, enters the middle layers of epithelial cells, and then

preferentially spreads outward into the tears. Similarly, movement of virus toward the cell body was directly observed and indicated that certain viral components are essential for transport to the nerve cell body and therefore establishment of latency.

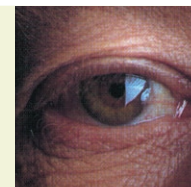
Understanding the mechanism of viral latency and reactivation and learning how virus is transported from the corneal epithelium to the nerve cell body or vice versa may lead to treatments that inhibit these processes and prevent recurrent disease and scarring. In future studies researchers will be able to examine the gene products that are essential to viral release from nerves, the genes involved in the spread of virus between the cells of the epithelium, and the transport machinery that mediates these movements.

#### **RIVER BLINDNESS**

Ocular onchocerciasis, commonly known as river blindness, occurs when a nematode worm infects the cornea. Although river blindness is rare in developed countries, it is the second leading infectious cause of blindness in the world. The disease begins with repeated bites from black flies that transmit the nematode larvae. The larvae settle in the skin, where they grow to adulthood, reproduce, and then release millions of microfilariae that travel through the skin and can infect various eye tissues, including the cornea. When the microfilariae die, a massive inflammatory response is observed in the eye. This invasion of immune cells leads to corneal swelling, loss of transparency, and eventually blindness. Development and growth of a microfilaria depends on a bacterium, *Wolbachia*, which lives within the parasitic worm. Treatment of river blindness with anti-parasitic

drugs previously was considered effective, because it reduced the number of microfilariae. However, destruction of the worms leads to the blinding inflammatory response—not due to the worms, but to the bacteria residing within the microfilariae. Upon the death of the parasite, these bacteria are released into the corneal stroma and directly cause the inflammatory reaction that results in blindness.

Using a mouse model of river blindness, scientists examined the inflammatory response in corneas subjected to extracts of worms that either were treated with doxycycline antibiotic, to kill the bacteria, or were untreated, and therefore contained living bacteria<sup>64</sup>. When corneas were subjected to bacteria-free worm extracts (i.e. treated with antibiotic), the researchers observed no significant corneal haze or swelling, indicating little or no inflammatory response. This finding indicates the *Wolbachia* bacterium may be a good target for treatment, not only because it causes the death of the nematode, but also because it can reduce or eliminate the host inflammatory response caused by the bacterium. **Further development of this treatment could revolutionize treatment of river blindness**, because the prompt destruction and removal of the *Wolbachia* bacteria should prevent the ensuing blindness associated with ocular onchocerciasis.



## Dry Eye

Dry eye is a disorder of the tear film due to tear deficiency or excessive tear evaporation that causes damage to the ocular surface. Symptoms of dry eye include burning, scratching, or stinging sensation. The condition is most often associated with aging and with diseases such as Sjögren's syndrome, diabetes, and inflammatory diseases of the eyelid (blepharitis, rosacea, and meibomian gland dysfunction). Dry eye can also be caused by environmental factors such as exposure to dry climates or heating and air conditioning systems with forced air. If left untreated, severe dry eye may lead to dehydration of the corneal epithelium, which in turn can cause ulceration and perforation of the cornea. Progress has been made in understanding of both the underlying causes of dry eye and in the structure and formation of tears.

### TEAR COMPONENTS

The external surface of the eye is covered by a thin tear film that lubricates and protects it from the external environment. Human tears that form this film consist of several proteins that are essential for its maintenance and proper function. Three of these proteins are packaged and secreted together by the lacrimal gland that produces tears. NEI-supported researchers have found that these proteins, lipocalin, lysozyme, and lactoferrin, strongly interact with each other as well as with lipids in tear film<sup>65,66</sup>. Knowledge of the structural basis for tear formation will lead to better strategies to treat dry eye. This debilitating condition often occurs in women as a result of the aging process or as a result of an autoimmune disease known as Sjögren's syndrome.

### SJÖGREN'S SYNDROME

The hallmark of Sjögren's syndrome is severe dry eye and dry mouth. In the eye, the function of the lacrimal gland, which produces the majority of the fluid in tear film, is severely compromised by infiltration of immune lymphocytes. The cause of this autoimmune response is thought to be a loss of tolerance to intracellular proteins that are not normally exposed on the cell surface. One possibility for the exposure to the immune system may be attributable to cell death. Another mechanism for exposing these proteins on the cell surface could occur during movement of these proteins through vesicles in lacrimal gland cells that shuttle the intracellular protein between membranous compartments<sup>67</sup>. This trafficking of proteins may be disrupted, thereby causing misdirection of normally intracellular protein to the surface of cells where they are available to stimulate attack by infiltrating lymphocytes. Thus, the immune system may exhibit tolerance to antigenic proteins normally found in the lacrimal gland, but that tolerance may be challenged by disruptions in normal physiologic mechanisms that ultimately may evoke an immune response.



## Wound Healing and Therapeutic Interventions

The epithelial cells of the cornea form a surface barrier that protects the underlying tissues from the external environment. When this layer is damaged, the epithelial cells normally respond quickly to close the wound and reform the barrier. In some cases, however, this response is defective, leading to the formation of persistent and painful corneal ulcers. Development of more effective treatments for this condition has been hampered by the limited information about the cellular and biochemical events that regulate corneal wound closure, and NEI-supported scientists have been working to increase our knowledge of these events.

### CORNEAL ULCERS

Scientists at the NEI discovered that an enzyme called Cdk5 plays a central role in regulating the migration of epithelial cells to close corneal wounds<sup>68</sup>. Cdk5 regulates Src, an enzyme which is known to control many aspects of cell movement. They found that overexpression of Cdk5 decreased the activity of the Src enzyme and inhibited cell migration. Conversely, drugs that inhibited Cdk5, promoted the accumulation of Src along the cell membrane, increasing the rate of cell migration and wound closure. **These findings suggest a new approach for treating persistent corneal ulcers and other conditions with impaired wound healing.** Animal studies are in progress to determine whether inhibitors of Cdk5 can safely be used in the eye to enhance wound healing.

### WOUND HEALING PROTEIN

The outermost tissue in the visual system is the corneal epithelium. If the integrity of this epithelium is compromised by trauma, the epithelium is rapidly activated to increase cell number (proliferation) and move cells to the damaged area (motility/migration) to close the wound and re-establish the barrier function. Scientists have already identified a number of biochemical components that contribute to the wound healing process. Recently, scientists found that a cell protein called pinin is able to modify a small number of genes involved with corneal cell proliferation and motility<sup>69</sup>. They found that cell motility genes were stimulated and cell proliferation and migration genes were inhibited by pinin. Since pinin increases cell adhesion and limits migration, it may be possible to enhance wound healing by inhibiting pinin's action. Alternatively, stimulation of pinin expression may play a role in suppression of tumors that develop due to abnormalities in cell adhesion pathways<sup>70</sup>. Additional research may help identify the key molecules associated with these pathways and may lead to treatments that enhance wound closure and more rapidly restore the epithelial barrier.



### **ANTI-INFLAMMATORY MOLECULES IN CORNEA WOUND HEALING**

Inflammation is a common immune response to injury and infection in the body. In the cornea, however, inflammation can cause extreme discomfort and result in vision loss. Nonetheless, the cornea retains a remarkable capacity for wound repair while actively suppressing an inflammatory response. Scientists have recently discovered that two lipids, lipoxin A4 (LXA4) and docosahexaenoic acid-derived neuroprotectin D1 (NPD1), are formed in the cornea and act as anti-inflammatory agents during corneal infection and wound healing<sup>71</sup>. Topical treatment with LXA4 and NPD1 in mice with corneal injuries increased the rate of tissue repair without impairing the recruitment of key immune leukocytes, which are normally associated with inflammation, into the wounded tissue. Moreover, a transgenic mouse that lacks these lipids exhibited delayed wound healing and attenuated leukocyte recruitment. The identification of these anti-inflammatory lipids in the cornea and their enhancement of wound healing by topical application suggest their use as therapeutic agents to overcome aberrant and damaging inflammatory responses in the eye.

### **REDUCING HAZE AND APOPTOSIS**

Excimer laser surgery is an FDA approved treatment for use in the correction of nearsightedness (myopia), farsightedness (hyperopia), and irregularities in the corneal surface (astigmatism). One surgical technique is known as photorefractive keratectomy or PRK. In PRK, the light energy of the laser removes precise amounts of the corneal tissue (also known as photoablation) to reshape the surface, thereby improving the cornea's refractive properties in focusing light on the retina. Although PRK is effective in reducing refractive errors, there is a small risk, particularly in patients with higher degrees of refractive error, of the development of corneal haze that reduces the transparency of the cornea. In spite of technological improvements in the excimer laser, the mechanism involved in development of corneal haze is not clear and treatment has had limited success.

However, a novel application using tissue from the amniotic membrane may offer improved outcomes with PRK. The amniotic membrane is the inner membrane of the placenta that surrounds a fetus during development. After birth this tissue is discarded. Researchers have previously reported the use of amniotic membrane in reconstruction of the corneal surface in patients with a variety of eye diseases. Because of the success of these procedures in promoting healing while reducing inflammation and scarring, scientists wanted to know whether transplantation of amniotic membrane after photoablation would reduce or eliminate corneal haze<sup>72</sup>. Animals received PRK in both eyes, and the cornea of one eye was subsequently covered with a dressing

or bandage of amniotic membrane. The uncovered cornea served as the control. Investigators found that the use of amniotic membrane at an early stage in the corneal healing process markedly reduced corneal haze in animals treated with the excimer laser. Investigators also found fewer apoptotic corneal cells (cells with characteristic features of programmed cell death) and fewer inflammatory infiltrates in the corneas covered with the amniotic membrane, suggesting suppression of apoptosis in those corneas. Although amniotic membrane has been used in corneal reconstruction, its use as a bandage or dressing to promote wound healing and reduce inflammation has now been demonstrated. The success of this technique may be useful in reducing corneal haze after PRK, but it also has important implications for use in enhancing corneal healing after damage by injury, disease, or infection.

### **CORNEAL TRANSPLANTATION**

Although most corneal transplants are successful, approximately 20 percent fail due to immunologic rejection of donor tissue. Researchers have found evidence that host tissues at the transplant site increase the concentration of certain molecules that are responsible for recruiting the immune and inflammatory cells that cause rejection. Specific inhibitors of these molecules may one day be used as topical agents to suppress the immune response and ultimately lead to greater success of grafts<sup>73,74</sup>.



## Cornea Structure, Function, and Genetics

The cornea must remain transparent in order for light to enter the eye and for images to be properly focused on the light-sensitive retina. The cornea is composed of an external five to six cell thick layer called the epithelium, a thicker stroma, and a single-layered endothelium. These layers must remain intact for the cornea to perform its role of protecting the inner structures of the eye. Much has been learned recently about the structure, function, and protection of the cornea.

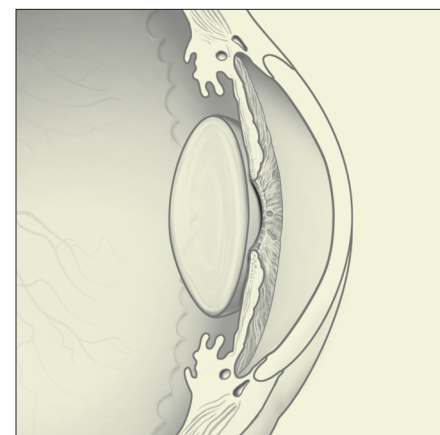
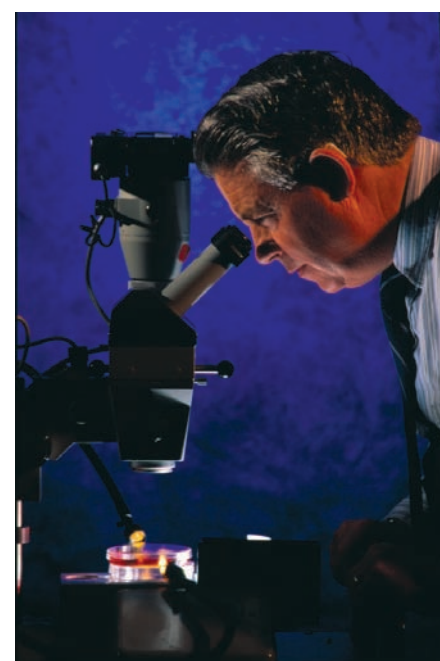
### PROTECTIVE MECHANISMS

Despite its exposure to cancer-causing UV radiation, the cornea rarely develops primary tumors. This is due to a unique mechanism that transports a molecule called ferritin, which protects DNA, into the nucleus of corneal epithelial cells. Although most cells have high cytoplasmic levels of the protein ferritin, corneal epithelial cells have high levels of ferritin in the nucleus. In this location, ferritin is highly effective in protecting DNA from UV-induced damage.

Proteins are synthesized in the cytoplasm of a cell. To move from the cytoplasm to the nucleus, substances must pass through holes or pores in the nuclear membrane. Large proteins that are too big to move through the pore by diffusion contain nuclear localization sequences that facilitate passage. Ferritin, which is a large protein, does not possess these sequences but nonetheless manages to infiltrate the nucleus. In examining this phenomenon, researchers have discovered a novel protein called ferritoid, specific for corneal epithelial cells, which binds to ferritin and carries it into the nucleus<sup>75</sup>. This discovery may provide a model for developing other molecules that could shuttle other protective substances to the nucleus of a cell. More knowledge about the mechanism of action of ferritoid may also provide clues for how the nuclear pore complex operates and may permit studies of ferritin's mechanism of action after penetrating the nuclear membrane.

### CORNEAL EPITHELIUM

While the physical basis for transparency of the corneal stroma is well studied, much less is known about the optical properties of the corneal epithelium. Interestingly, the corneal epithelium accumulates surprisingly high proportions of just a few proteins. These abundant proteins of the corneal epithelium often differ in different species. For example, NEI intramural investigators recently examined the protein composition of the zebrafish corneal epithelium<sup>76</sup>. A protein called gelsolin was found to account for approximately 40-50 percent of the water soluble proteins. Gelsolin is a large protein that regulates the cellular cytoskeleton and has important roles in cellular motility among other processes. The finding that gelsolin is the major soluble protein of zebrafish corneal epithelial cells suggests a novel role for this protein in vision. Interestingly, a single-base mutation of the human gelsolin gene has been shown to cause a form of corneal dystrophy, making the high concentration of gelsolin in the zebrafish of great medical interest.



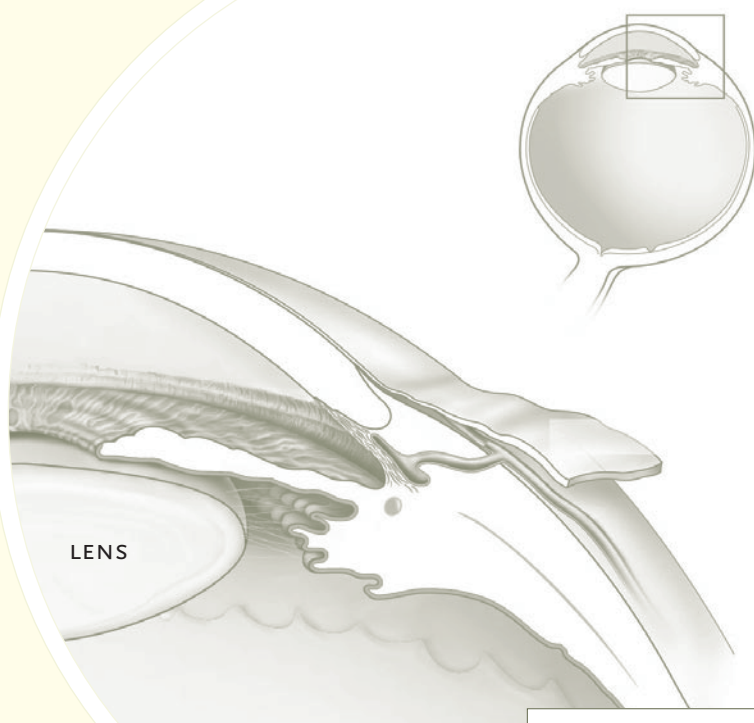
## **CORNEAL MATURATION**

Corneal diseases are plentiful and difficult to treat. For example, keratocornus is a progressive corneal disease that results in a thin, bulging, conically shaped cornea that can cause severe visual impairment. Other corneal diseases are associated with blistering and poor adhesion of corneal epithelial cells, the outermost cell layer of the cornea. Many of these diseases progress to a point where the cornea is no longer viable. However, unlike intraocular lens implants to treat cataracts, corneal implants are much less successful and may become opaque with time. There are also no artificial corneas available yet. Thus, knowledge of the mechanisms regulating the development and maintenance of the cornea is of medical importance.

Previous studies have shown that p63, a transcription factor involved in growth and specialization of epithelial cells, is expressed in cornea cells. Transcription factors are proteins that recognize and bind to specific regions of a gene that regulate its expression or activity. A recent study has found that during development corneal cells also express a protein called high mobility group protein N1 (HMGN1)<sup>77</sup>. Both p63 and HMGN1 are expressed in precisely the same set of corneal cells with a pattern that changes during corneal cell development. Loss of HMGN1 results in corneal epithelium thinning, loss of proper stratification of corneal cell types, blistering, and an abnormal rate of growth of epithelial cells. Moreover, cornea lacking HMGN1 show premature changes in p63 expression. The finding that p63 and HMGN1 are connected with aspects of corneal biology provides new targets for investigations that may ultimately have clinical relevance.

## **GENE FOR CORNEAL DYSTROPHY**

Francois-Neetens mouchetée fleck corneal dystrophy (CFD) is a rare genetic corneal dystrophy characterized by numerous small white flecks scattered in the cornea. Vision researchers have identified mutations in a gene called phosphatidylinositol-3-phosphate 5-kinase, type III (PIP5K3) that cause CFD. PIP5K3 is part of a family of enzymes that help regulate the formation and intracellular location of lipid products<sup>78,79</sup>. It is thought that mutations in the gene disrupt the transport of lipids within the membranes of corneal cells, resulting in the characteristic flecks that appear in the corneas of people with CFD. Besides providing insight into the pathophysiology of CFD, this discovery provides a new avenue of exploration into both corneal biochemistry and physiology.



## Advances in Lens and Cataract Research

CATARACT, AN OPACITY OF THE LENS OF THE EYE, interferes with vision and is the leading cause of blindness in developing countries. In the U.S., cataract is also a major public health problem. An estimated 26.6 million Americans over age 40 have cataract or have had surgery to remove the lens opacification. Currently, cataract surgery accounts for 60 percent of vision-related Medicare expenditures. However, by 2020 researchers estimate that 39.6 million Americans will be affected by cataract<sup>80</sup>. The enormous economic burden of cataract will only worsen as the American population ages. The major goals of this program, therefore, are to determine the causes and mechanisms of cataract formation, to search for ways to slow or prevent the progression of cataract, and to develop and evaluate new diagnostic and therapeutic techniques in cataract management.





## Structure and Transparency

The lens contains two cell types: metabolically active epithelial cells and quiescent fiber cells. Throughout life, the lens continues to grow, with epithelial cells dividing and differentiating into fiber cells. During differentiation epithelial cells lose their intracellular metabolic structures and accumulate high concentrations of crystallin proteins to produce the high refractive index required for transparency. Physical and chemical changes in lens proteins may occur due to normal aging or other processes that result in a loss of transparency or clarity. Scientists continue to increase their understanding of the changes that occur during aging with the aim of being able to prevent cataract formation.



SIMULATION  
View seen with Cataract  
(Normal Vision view on page 8)

### PROTECTION OF THE LENS

To maintain transparency, the lens uses a chaperone protein to protect other proteins when the cell is subjected to stress. In the lens,  $\alpha$ -crystallin, a protein belonging to a family of chaperones is composed of two subunits that also function as chaperones. Further investigation of the protective roles of these subunits in epithelial cells led scientists to test their ability to protect human epithelial cells from apoptosis (programmed cell death)<sup>81</sup>. They found that the subunit  $\alpha$ A-crystallin protected the cells from a variety of substances that are known to cause apoptotic cell death. They also found that  $\alpha$ A-crystallin is two to three times more effective than the other subunit in protecting animal lens epithelial cells grown in the laboratory from stress-induced cell death.

### PROTEIN INTEGRITY AND LENS TRANSPARENCY

A cause and effect relationship has been difficult to decipher in cataract formation because the end-result is in most cases far removed in time from the initial insult. It has long been recognized that lens transparency results from the very high concentration of soluble proteins, the crystallins, within a specialized lens fiber cell. It has also been long known that there is little turnover of proteins within these cells. An adult lens contains proteins synthesized at the earliest stages of embryological development, making fiber cell proteins especially susceptible to the effects of aging. The normal lens counteracts the aggregation of the soluble lens crystallins that occurs in aging through the function of  $\alpha$ -crystallin, which acts as a molecular chaperone, preventing the unfolding and aggregation of proteins. New research provides data linking the formation of high molecular weight crystallin complexes with diminished chaperone activity<sup>82</sup>. Scientists examined lenses during aging and cataract formation. They found that as  $\alpha$ -crystallin acts to prevent the deleterious effect of aggregate formation by binding to other proteins,  $\alpha$ -crystallin itself becomes incorporated into an aggregate. Since  $\alpha$ -crystallin strongly binds to the lens fiber cell membrane, it becomes a vehicle for complexes to accumulate at the membrane, allowing further damaging physiological effects that may accelerate cataract formation. These new data also showed that the  $\alpha$ -crystallin in this membrane-bound aggregate has a significantly diminished capacity to function as a chaperone, indicating that its protective effect has been neutralized.



### HEAT SHOCK PROTEINS IN THE LENS

In the lens,  $\alpha$ -crystallin has a dual function: it accumulates in fiber cells in high concentrations to produce the high refractive index needed for transparency. Like other small heat shock proteins that react to cellular stress,  $\alpha$ -crystallin has some flexibility in function depending on the cell environment. New data suggest that under low stress,  $\alpha$ -crystallin is maintained in a multi-subunit complex<sup>83,84</sup>. Under conditions of high stress,  $\alpha$ -crystallin breaks into smaller sub-units. This shift coincides with the formation of a high capacity form increasing the efficiency of the  $\alpha$ -crystallin chaperone function due to an increase in surface binding capacity. This chaperone function protects against clouding of the lens due to protein aggregation. **Improving our understanding of this protective role of  $\alpha$ -crystallin may one day lead to the means to prevent cataract.**

### FIBER CELL FORMATION AND MAINTENANCE

The lens is a dense, compact structure containing two cell types: metabolically active epithelial cells and quiescent fiber cells. Throughout life, the lens carries out a process of continued growth with epithelial cells dividing and differentiating into fiber cells. As epithelial cells differentiate into fiber cells, they become denuded of organelles such as the nucleus and mitochondria. Elimination of organelles is critical because they would interfere with the refractive index and clarity of

the lens. It has been suspected that epithelial cells “borrow” enzymes involved in programmed cell death, or apoptosis, to mediate organelle destruction. Apoptosis is a normal biologic process that guides an orderly destruction of cells that are no longer functional or needed. In a recent study, NEI-supported scientists have shown that specific forms of caspases, a group of protein degrading enzymes critical to dismantling organelles during apoptosis, are also involved in fiber cell formation as well<sup>85</sup>. This study defines a critical step in how fiber cells are formed and is leading to further investigation into whether alterations in caspase enzymes play a role in cataract formation.

### MAINTAINING TRANSPARENCY

Scientists have previously found that the lens uses proteins involved in a biological process called apoptosis or programmed cell death to rid lens fiber cells of their organelles. Vision researchers have now discovered a process that allows these apoptotic processes to occur without causing the death of fiber cells. They have termed the process, Apoptosis-related Bcl-2 and Caspase-dependent (ABC) differentiation<sup>86</sup>. In this process, a number of proteins that normally lead to cell death such as caspases—proteins that break-down internal cellular structures—are used to signal the beginning of differentiation. The expression of cell death proteins is balanced by the simultaneous induction of pro-survival molecules such as bcl-2, a protein that binds to cell death proteins and inhibits further

damage or death to fiber cells. This allows lens fiber cells to achieve the high level of transparency needed for clear vision. The discovery of ABC differentiation in the lens will allow researchers to better understand lens cell renewal and determine whether faulty mechanisms in this process might lead to cataract formation.

### LENS PROTEIN AGGREGATION

One hypothesis of cataract formation is that  $\alpha$ -crystallin activity decreases as a consequence of age allowing proteins to coalesce into light scattering aggregates that lead to opacification. Recently, a group of NEI scientists found that aging  $\alpha$ -crystallin becomes modified by the addition of chemical compounds known as phosphate groups<sup>87</sup>. The scientists found that the addition of phosphate groups to strategic places along the  $\alpha$ -crystallin protein altered its structure and increased its propensity to aggregate with other proteins. Rather than preventing lens cell proteins from aggregating, the addition of these phosphate groups becomes part of the problem. This study provides an elegant approach to understanding normal lens physiology and identifying how physiological changes as a result of aging make the lens vulnerable to cataract formation.

## Lens Development and Communication

The development of the lens occurs early in the development of the eye. Identifying developmental genes and their products is essential to the understanding of the hierarchy controlling ocular development and will enhance our knowledge of the molecular basis of congenital diseases of the eye, thereby opening the possibility of future interventions. Studies of communication between cells in this unique tissue also enhance our understanding of the mechanisms of intercellular communication in general. Fiber cells lack the infrastructure for metabolism, and therefore they must depend on epithelial cells for their sustenance. The lens utilizes gap junctions, proteins in the cell membrane that allow the exchange of small molecules, to meet their unique requirements.

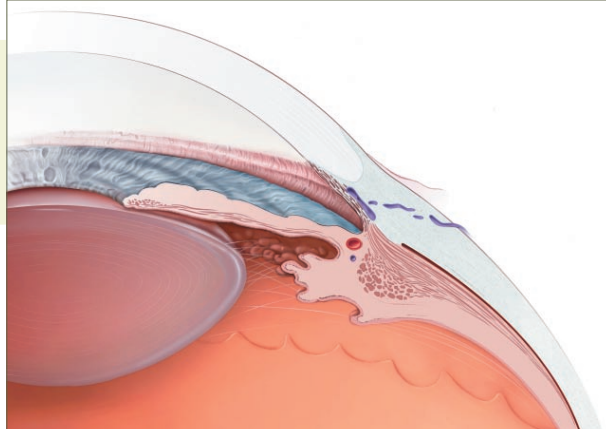
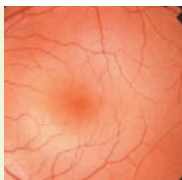
### EARLY EYE DEVELOPMENT

Development of the lens is one of the earliest events in the development of the eye. Scientists have been attempting to determine the genes that control lens development. Studies have shown that Pax-6 is a master gene that controls the expression of a number of other critical genes. Two critical genes that have recently been identified are Six3 and Grg5<sup>88</sup>. Without the expression of these two genes, the development of the lens is stopped and crystallin-synthesizing cells fail to form. These findings add to our understanding of the overall control of lens and eye development.

### VASCULAR REMODELING IN THE LENS

During development, the immature lens and pupillary membrane are nourished by blood vessels that regress as the eye matures. However, in some cases, these vessels fail to regress, causing persistent fetal vasculature syndrome (PFVS), a condition that leaves the lens opaque. A recent study of patients with PFVS found persistent expression of pigment epithelial-derived factor, a protein associated with blood vessel growth. The study also found evidence to suggest that beta and gamma crystallin, two common lens proteins that help give this tissue its transparency, also function in helping to create and maintain these developmental vessels<sup>89</sup>. This study lends clarity to the basis of this blinding, congenital eye disease and offers new avenues to develop treatments that promote regression of these developmental blood vessels.





### **LENS DEVELOPMENT AND RETINA**

The development of the human eye requires that diverse tissues differentiate and migrate in a coordinated fashion. Researchers have been unclear how this developmental coordination is achieved. A recent study has found that the PITX3 gene, which is associated with a variety of congenital eye diseases that affect the lens and cornea, is involved in coordinated development of the lens and retina<sup>90</sup>. Investigators prevented expression of the PITX3 gene in zebrafish and found abnormalities in lens and retinal development. Retinas of these fish had degenerated cellular nuclei and fewer neuronal cells, suggesting that this lens protein exerts influence on retinal cell differentiation and development. This study provides a new animal model to further understand the developmental pathways that coordinate diverse tissue development within the eye.

### **LENS CELL COMMUNICATION AND GAP JUNCTIONS**

Throughout the life-time of an individual, the lens carries out a process of continued growth with epithelial cells dividing and differentiating into fiber cells. Unlike most other tissues, the lens is not nourished by its own blood supply and requires an extensive communication system made up of channels called gap junctions that take up nutrients. Mice with deletions in the proteins that make up gap junctions develop cataracts, as well as developmental abnormalities. Detailed studies in mice in which a deleted gap junction protein is replaced by a related but different gap junction protein have given new insight into the relationship between cell division, differentiation, and communication<sup>91</sup>. These studies highlight the significance of the intricate communication system of the lens and its requirements for maintaining lens transparency. They also provide additional information that may aid in preventing the loss of transparency.

### **LENS CELL COMMUNICATION**

Gap junctional communication joins the cells of the lens into a functional unit in which the metabolically active epithelial cells can control the environment needed in the fiber cells to maintain transparency. Previous studies have shown that mutations in genes encoding gap junction proteins lead to dysfunctional proteins resulting in hereditary cataracts. Scientists have now identified two different types of gap junction proteins in the lens known as connexins. They have found that mice with deletions in genes encoding connexins develop cataracts as well as other developmental abnormalities. These studies have revealed differing roles for the two gap junction proteins, one is primarily responsible for maintaining transparency and the other is needed for growth. Researchers also found that a mutation in the growth associated gene resulted in hereditary cataracts in humans<sup>92,93</sup>.



## Prevalence and Risk Factors

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Studies of the natural progression, incidence, and prevalence of diseases allow the investigation of genetic, physiological, or environmental factors that increase the risk of development or progression of the disease. These studies are also essential in providing clues to laboratory scientists investigating the underlying mechanisms involved in the disease process.

### SMOKING

Smokers are known to be at increased risk of developing cataracts. **A large prospective epidemiologic study following a group of almost 21,000 male physicians for 14 years has determined that those who stop smoking reduce their risk of cataract<sup>94</sup>.** At least part of this decreased risk is due to less lifetime cigarette usage, although the investigators provide evidence to suggest that some smoking-related damage to the lens of the eye is reversible. These findings provide additional evidence of the benefits derived from smoking cessation.

### THE AGE-RELATED EYE DISEASE STUDY (AREDS)

The AREDS, sponsored by the National Eye Institute, is an ongoing multi-center study of the natural history of cataract and age-related macular degeneration. AREDS has been designed in part to search for clues about the etiology of cataract and possible strategies for intervention. Data were collected at entry on a wide range of possible risk factors for cortical and nuclear cataracts, two of the most common types of cataract. Results from the study reinforce a growing consensus that smoking increases the risk of development of nuclear cataract and that higher levels of sunlight exposure increase the risk of cortical cataract<sup>95</sup>. The identification of these potentially modifiable risk factors for cataract reinforces public health recommendations to avoid smoking and decrease exposure to sunlight.



## Advances in Glaucoma and Optic Neuropathies Research

GLAUCOMA is a group of eye disorders that share a distinct type of optic nerve damage, which can lead to blindness. Elevated intraocular pressure (pressure inside the eye) is frequently, but not always, associated with glaucoma. Glaucoma is a major public health problem and the number one cause of blindness in African Americans. Approximately 2.2 million Americans have been diagnosed with glaucoma and the prevalence of the disease will rise to a projected 3 million by 2020<sup>6</sup>. Most of these cases can be attributed to primary open angle glaucoma, an age-related form of the disease. NEI activities in glaucoma research are directed toward understanding the mechanisms of the disease through basic research, identifying risk factors, and preventing blindness.

## Clinical Trials

Much of the medical research effort is aimed toward developing sufficient understanding of glaucoma so that preventative strategies can be developed or safe and effective therapies can be designed. Evaluations of new treatments, as well as careful comparisons of the efficacy of commonly prescribed treatments are important areas of progress in clinical research related to glaucoma.

### EARLY MANIFEST GLAUCOMA TRIAL

The Early Manifest Glaucoma Trial was designed to compare the effect of immediate therapy to lower the intraocular pressure (IOP) versus late or no treatment on the progression of newly detected open-angle glaucoma<sup>97</sup>. The study followed 255 patients, aged 50-80 years, with early stage glaucoma in at least one eye. One group (129 patients) was treated immediately with medicines and laser to lower eye pressure, and a control group (126 patients) was untreated. Both groups were followed carefully and monitored every three months for early signs of advancing disease, using indicators that are extremely sensitive for detecting glaucoma progression. Any patient in the control group whose glaucoma progressed was immediately offered treatment. After six years of follow-up, scientists found that progression was less frequent in the treated group (45 percent) than in the control group (62 percent), and occurred significantly later in treated patients. Treatment effects were also evident in patients with different characteristics, such as age, initial eye pressure levels, and degree of glaucoma damage. In the treated group, eye pressure was

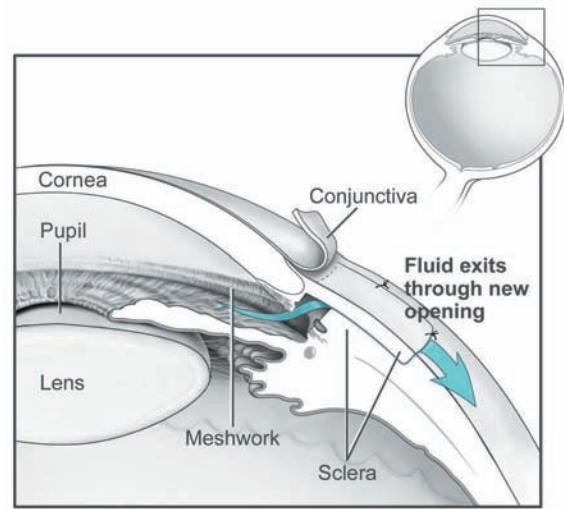
lowered by an average of 25 percent. **This finding supports the medical community's emerging consensus that treatment to lower pressure inside the eye can slow glaucoma damage and subsequent vision loss.**

### THE OCULAR HYPERTENSION TREATMENT STUDY

NEI-supported researchers have discovered that eye drops used to treat elevated IOP are effective in delaying the onset of glaucoma in people at high risk for the disease<sup>98</sup>. The Ocular Hypertension Study (OHTS) found that pressure-lowering eye drops reduced by more than 50 percent the development of primary open-angle glaucoma, the most common form of glaucoma and one of the nation's leading causes of vision loss. The OHTS study also identified several significant risk factors that were associated with the development of glaucoma in study participants. These included personal risk factors, such as older age and African descent, as well as ocular risk factors, such as higher eye pressure, certain characteristics in the anatomy of the optic nerve, and thinness of the cornea. These results mean that treating people at higher risk for developing glaucoma may delay or possibly prevent the disease.

### AFRICAN AMERICANS WITH GLAUCOMA

**The prevalence of glaucoma is three times higher in African Americans than in non-Hispanic Whites.** Additionally, the risk of visual impairment is much higher and the age of onset is earlier than in Whites. About 70 percent of glaucoma cases are associated with a history of elevated intraocular pressure (IOP). An NEI-supported follow-up study to the Ocular Hypertension Treatment Study (OHTS) found that early treatment of elevated IOP reduces the risk of developing glaucoma in African Americans<sup>99</sup>. Of the participants in the treatment arm of the study, 8.4 percent developed glaucoma whereas 16.1 percent in the observation group developed the disease. The OHTS follow-up study reinforced the previous finding that certain biological characteristics of the eye including corneal thickness are helpful in predicting who will likely develop glaucoma and who will benefit from therapy.





## Neuroprotection

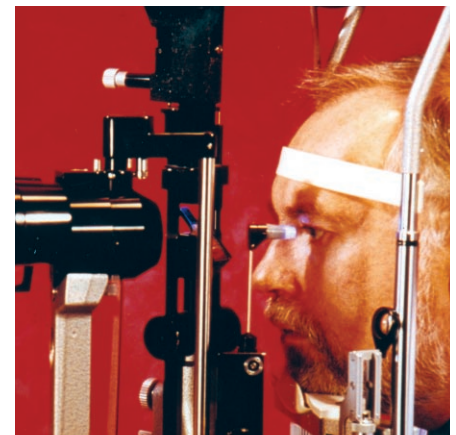
Among the newest therapeutic strategies for reducing or eliminating damage to nerve cells caused by neurodegenerative diseases is that of neuroprotection. Among the approaches being studied are stem cell therapy, gene therapy, vaccination, and the use of neurotrophic factors. Scientists are making progress in improving retinal ganglion cell (RGC) survival using these approaches.

### NEUROPROTECTIVE THERAPY IN GLAUCOMA MODEL

A hallmark of glaucoma is the death of RGCs, which can lead to catastrophic vision loss. Insights gained from a series of NEI-supported studies in animal models have recently culminated in an experimental gene therapy for glaucoma that might one day augment or replace existing treatments to better protect RGCs<sup>43</sup>. Previous NEI studies have found evidence that elevated intraocular pressure deprives RGCs of brain-derived neurotrophic factor (BDNF), an endogenous protein that is crucial to RGC survival. Although RGCs produce some BDNF, levels are further enhanced by adjacent cells of the lateral geniculate nucleus, which produce and transport BDNF to RGCs. Ocular injections of BDNF in rodent models of glaucoma have improved RGC survival. However, due to the relatively short half-life of this protein, the need for frequent ocular injections would not bode well in treating a chronic disease like glaucoma. To overcome this hurdle, **NEI-supported researchers recently used gene therapy in rodent models of glaucoma to transfect RGCs with the gene that encodes BDNF, providing a lasting and direct supply of this essential protein.** In short-term experiments, treated eyes had a marked improvement in RGC survival than those of control animals. Further NEI-supported laboratory work is evaluating whether gene therapy with BDNF provides long-term benefit and whether gene delivery with other neurotrophic agents, alone or in combination with BDNF, improves RGC survival.

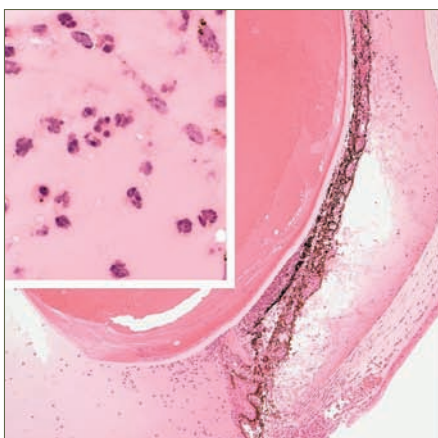
### NEUROPROTECTION IN GLAUCOMA

In a highly novel finding, researchers have found that high-dose radiation with bone marrow transfer prevents the loss of RGCs in an animal model of glaucoma<sup>100</sup>. The neuroprotection offered by this procedure was complete, highly reproducible, and lasting. Normally, by 12-14 months, these glaucoma susceptible mice have complete RGC loss. At 14 months, treated mice had no detectable signs of disease. Although the mechanism that offers neuroprotection is not yet known, researchers speculate that it is due to radiation, because the transferred bone marrow was genetically identical to the original bone marrow the mice were born with. This treatment protocol offers a tool to understand neurodegeneration and, with refinement, could have important implications for the treatment and prevention of neurodegenerative diseases.



SIMULATION  
View seen with Glaucoma  
(Normal Vision view on page 8)





## Genetics and Cell Biology

Vision loss from glaucoma is associated with the degeneration and death of RGCs. This degeneration is most commonly associated with elevated intraocular pressure; however, in some patients an elevation in pressure is not evident on clinical examination. The genetic and cellular bases for the development of glaucoma either with or without an accompanying increase in intraocular pressure are areas of active investigation and considerable progress toward understanding these mechanisms has been reported.

### MYOCILIN IN AQUEOUS HUMOR

NEI-supported scientists have identified mutations in a gene called myocilin that is linked to a rare, inherited form of glaucoma. The myocilin protein is found in cells of the trabecular meshwork. It is also found in other eye tissues, but researchers wanted to know whether myocilin was also present in the aqueous humor, the fluid involved in elevated IOP. Analysis of human, monkey, and cow eyes found that myocilin was present in the aqueous humor of all three species. Further investigation found that the protein was larger than expected and was hydrophobic or unable to mix with water. The scientists also found that myocilin tightly adheres to micro filters that are present in the trabecular meshwork. These same filters become obstructed in glaucoma, suggesting that mutant myocilin may contribute to IOP<sup>101</sup>.

### MECHANISM OF MYOCILIN ACTION

Elevated IOP is believed to be an important factor in the majority of cases of glaucoma. It results from an imbalance in the inflow and outflow of aqueous humor. A major breakthrough in the study of glaucoma came with the mapping and identification of mutations in myocilin, a gene associated with an inherited form of glaucoma. NEI-supported scientists used cell cultures to show that mutant forms of myocilin do not fold properly, resulting in abnormal shaped proteins that clump within the cells of the trabecular meshwork. The abnormal protein accumulates within the cell eventually causing the cell to die. As the trabecular meshwork cells die, the outflow tissue becomes dysfunctional. Importantly, the scientists also found that myocilin proteins displayed temperature sensitivity and when the temperature was lowered, they folded properly. Proper folding led to a reversal of the cellular pathology and rescue of the cells thus suggesting a new therapeutic avenue involving some method of periodic cooling treatments to the trabecular meshwork<sup>102</sup>.



### MYOCILIN AND GLAUCOMA

Previous studies have found that the myocilin gene is expressed in the cells of the trabecular meshwork (TM), and mutations in the myocilin gene were found to cause a rare, inherited, early onset form of glaucoma. However, the consequence of increased myocilin has not been studied in vivo. To gain better insight, scientists developed a mouse model genetically engineered to secrete large quantities of human myocilin into the aqueous humor. Surprisingly, IOP did not increase despite the fact that levels of myocilin in their aqueous humor were five times that in the normal human eye<sup>103</sup>. In parallel experiments, mice were genetically engineered to express a mutated form of myocilin. The mutated protein product caused a detrimental accumulation in the cells of the TM and was not found in aqueous humor. This study has documented that high levels of the normal myocilin protein in the aqueous humor do not necessarily produce glaucoma. Conversely, the finding that mutated myocilin accumulates within the TM, offers an explanation for the difficulty these cells have in regulating IOP. This accumulation is thought to be responsible for the subsequent development of glaucoma.

### MYOCILIN EXPRESSION IN DROSOPHILA

Progress in glaucoma research has been somewhat hampered by the lack of a naturally occurring animal model that would provide insight into the biological mechanisms of the disease. Nonetheless, scientists have been using the common fruit fly, *Drosophila melanogaster*, as a genetic discovery system to better understand the role of the myocilin gene, which has been implicated as a cause of a rare, inherited form of glaucoma<sup>104</sup>. When genetically modifying myocilin so that it over-expresses, scientists found that the flies had enlarged eyes, indicating an imbalance in aqueous humor production and outflow.

The scientists then compared the genes expressed in the eyes of mutant flies to their normal counterparts. A number of genes were found to be altered, including aquaporin-4 and cytochrome-P450, which have been implicated as possible candidate genes in human glaucoma. In order to further validate their findings, the investigators next used an organ culture system created from donated human eyes. They found that the expression of one of the gene products—called Swiss Cheese in the fruit fly and neuropathy target esterase in humans—was markedly enhanced in both systems. This gene is thought to contribute to nerve cell degeneration. Future investigation using these model systems may help identify genes and physiological cascades that contribute to the disease in common forms of glaucoma.

### GENE ASSOCIATED WITH A FORM OF GLAUCOMA

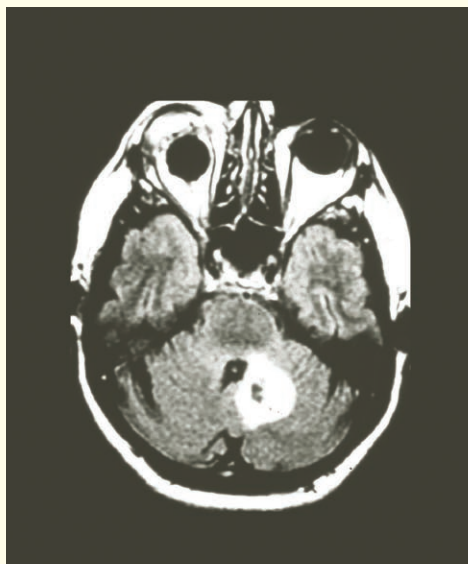
**Scientists have recently identified a human gene, OPTN, that is linked to a disease known as “low-tension” glaucoma<sup>105</sup>.** In patients with this form of the disease, clinicians are unable to detect pathological elevations of intraocular pressure. Four separate mutations in this gene were identified in families in which “low-tension” glaucoma was known to be inherited. Further screening of glaucoma patients suggested that mutations in OPTN may be a risk factor for “low-tension” glaucoma patients. This gene encodes the protein optineurin, which is expressed in a number of tissues including the brain and retina. Optineurin has been shown to interact with other brain proteins such as huntingtin, the protein responsible for Huntington’s disease and therefore may have a significant neurological function. Other studies suggest that optineurin participates in a signal transduction pathway involving tumor necrosis factor-alpha, a factor that is believed to increase the severity of optic nerve damage in glaucoma. Increasingly, scientists have viewed protecting the optic nerve as the key to treating the disease. The identification of OPTN as a glaucoma gene provides a tool to study the biochemical pathways leading to optic nerve degeneration, as well as giving insight into designing neuro-protective strategies.

### **EFFECT OF INTRAOCULAR PRESSURE ON BRAIN NEURONS**

Although elevated IOP has been associated with vision loss in glaucoma, experimental studies have not yet shown definitively that IOP causes glaucoma. Since all current treatments attempt to slow the progressive loss of neurons by reducing IOP, establishing this relationship would justify the continued use of these therapeutic strategies. Scientists now have evidence that increases in intraocular pressure have a profound effect on ganglion cell survival. Optic nerve fibers from RGCs connect to neurons in a part of the brain called the lateral geniculate nucleus (LGN). Neurons from the LGN then relay this information to the visual cortex for processing. Using a primate model of glaucoma, scientists showed that even relatively moderate elevations of intraocular pressure cause loss of LGN neurons over an extended period of time<sup>106,107</sup>. These data demonstrate that chronic elevation of intraocular pressure has a neurodegenerative effect on neurons critical for the integration and transmission of visual information and point to areas for future research to prevent or treat this damage.

### **MULTIGENIC AND ENVIRONMENTAL INFLUENCES IN GLAUCOMA**

Glaucoma is thought to result from a complex interaction of chronic environmental exposure to various risk factors and a multigenic predisposition to the disease. Identifying the genetic basis of complex eye diseases like glaucoma is a major research goal of the NEI. Because increased IOP is often associated with the development of glaucoma, investigators with the Beaver Dam Eye Study (BDES) performed a complex analysis of 2337 individuals from 620 families to determine whether a genetic predisposition to increased IOP exists. The BDES is a long-term prospective epidemiologic study of a large population in Beaver Dam, Wisconsin designed to collect information about age-related eye diseases. The study authors found that increased IOP correlates with multigenic and environmental influences<sup>108</sup>. The authors also found two new genetic loci on chromosomes 6 and 13. This study confirms the multigenic nature of glaucoma and suggests new areas of investigation to identify the genes that confer risk to the disease. In a parallel study that examined possible environmental findings, the BDES investigators found significant, direct correlations between elevated blood pressure and elevated IOP. Although further investigation is needed, this finding suggests the possibility that treatment to lower blood pressure might also reduce the risk of developing glaucoma. In tandem, these studies add to our understanding of environmental and genetic risk factors for glaucoma.



## Advances in Strabismus, Amblyopia, and Visual Processing Research

The Strabismus, Amblyopia, and Visual Processing Program supports clinical and laboratory research on visual development, neural processing, eye movement, and other disorders involving output of the retina and other portions of the brain that serve vision. Knowledge of the normal visual system provides a foundation for understanding the causes of impaired vision and developing corrective measures.





## Amblyopia

Amblyopia is the medical term used when the vision in one eye is reduced because the eye and the brain are not working together properly. The eye itself looks normal, but it is not being used because the brain is favoring the other eye. This condition is also sometimes called lazy eye. Amblyopia is a common cause of visual impairment in childhood. The condition affects approximately 2 to 3 out of every 100 children<sup>109,110</sup>. Unless it is successfully treated in early childhood, amblyopia usually persists into adulthood, and is the most common cause of monocular (one eye) visual impairment among children and young and middle-aged adults.

### TREATMENT OPTIONS

Eye patching has been a mainstay of treatment for children with amblyopia. Patching the stronger eye forces the use of the weaker eye and can improve or, if caught early, completely reverse the condition. Two patching regimens are commonly prescribed in clinical practice. One is minimal occlusion for two hours per day and the other is use of occlusion for six or more hours per day. Until recently, there were no data available to favor the use of one regimen over the other and compliance with patching varied widely. To address the clinical issue of the optimal number of patching hours for moderate amblyopia, a clinical trial comparing two hours versus six hours of daily patching for children with moderate amblyopia was conducted by the Pediatric Eye Disease Investigator Group (PEDIG), a network of eye care professionals involved in determining the best treatments for various eye problems in children. The results from this clinical trial revealed that patients in both groups showed substantial improvement in the eye with amblyopia<sup>111</sup>. At four months, 79 percent of patients in the two-hour patching group and 76 percent of patients in the six-hour patching group had improved by two or more lines on the eye chart. Children who wear patches often feel socially stigmatized, making the treatment an emotionally uncomfortable experience. This often interferes with compliance as children remove the patch to avoid the associated psychological burden. However, the two-hour regimen will allow children to wear the patch in the privacy of their homes.

In a parallel study conducted by PEDIG investigators, children with severe amblyopia who were less than 7 years of age were treated with either full-time patching or 6 hours of patching in combination with one hour of near work like reading or coloring. Researchers found that 6 hours of patching with an hour of near work was as effective in improving visual acuity as full-time patching<sup>112</sup>. Both of these studies should lead to better compliance with treatment for children with amblyopia. **These results will change the way doctors treat moderate and severe amblyopia and make an immediate difference in treatment compliance and the quality of life for children with this eye disorder.**



### AMBLYOPIA TREATMENT STUDY

NEI-supported researchers found that Atropine eye drops work as well as patching the eye in the treatment of amblyopia<sup>113</sup>. Atropine is used to blur vision in the stronger eye in order to force use of the weaker eye. Atropine offers an alternative to eye patching for children who do not want to wear eye patches. This research finding may lead to better compliance with treatment and improved quality of life in children with this eye disorder. Patients will continue to be followed in this study to better assess the long term effects these treatments have on visual acuity.

### Childhood Screening for Common Eye Diseases

Healthy vision is an important part of a child's success in school. A great deal of classroom instruction is conveyed visually through books, computer screens and chalkboards. Children who enter school with eye diseases or visual impairments are at a distinct disadvantage when encountering visually-based instruction. Childhood visual impairment can also result in developmental delays, the need for special education programs, social services and a lifetime of irreversible visual impairment. It is estimated that 20 percent of preschool children ages 3-4 have a treatable eye condition. While many states are developing guidelines for preschool screening programs, none of the commonly used vision tests have been evaluated in a research-based environment to establish their effectiveness.

### VISION SCREENING TESTS FOR PRESCHOOLERS

Results from the NEI-sponsored Vision in Preschoolers (VIP) Study found that 11 commonly used screening tests vary widely in identifying children with symptoms of common childhood eye conditions such as amblyopia, strabismus, and significant refractive error. When the best tests are used by highly skilled personnel in a controlled setting, approximately two-thirds of children with one or more of the targeted disorders were identified. These better tests were able to detect 90 percent of children with the most severe visual impairments. **The VIP study will provide state and local agencies with data to select the most effective vision screening exams that are currently available.** The VIP study will also help ensure that more children are detected and treated at an early stage when therapy is most effective<sup>114</sup>.



## Nerve Cell Regeneration

A fundamental issue in neuroscience has been the inability of some nerve cells to regenerate. Following injury or disease, neurons in the central nervous system (CNS) have a limited regenerative capacity, unlike nerve cells in the peripheral nervous system. Researchers have been engaged in developing therapies that overcome this limitation, so that the deleterious effects of many neurologic diseases and central nervous system (CNS) injuries might be reversed or greatly improved.



### STIMULATION OF NERVE CELL REGENERATION

NEI-supported researchers provoked nerve cell regeneration by activating a nerve cell's natural growth capacity and using gene therapy to suppress the effects of growth-inhibiting factors<sup>115</sup>. The researchers injured the optic nerves of rats and then caused an inflammatory reaction in the lens of the eye of the same animal. Previous work has shown that inducing inflammation actually stimulates immune cells called macrophages to release growth factors. These growth factors activate genes in retinal cells causing new nerve fibers (known as axons) to grow into the optic nerve. In an attempt to enhance this growth, the researchers added a gene therapy technique that effectively removed the inhibitory factors that block nerve fiber growth. Although vision was not restored, this combined approach stimulated nerve cell regeneration three times greater than prior attempts. Regeneration of the mature CNS would provide an opportunity to treat blindness and other neurologic diseases.

### DEVELOPMENTAL SWITCH

Nerve cells typically have two types of extensions that arise from their cell bodies. Axons are normally quite long and extend over considerable distances. Dendrites are much shorter and extend very short distances from the cell body. The inability of CNS neurons to regenerate is largely due to the failure of their axons to re-grow. It is believed that the inability of CNS axons to regenerate is due to the presence of a non-permissive microenvironment, containing factors that inhibit regeneration. Although these may be important factors, nerve cell interactions may play an inhibitory role as well. Recent work has shown that the developmental state of nerve cells may also play a role in the ability of CNS neurons to regenerate.

It is known that postnatal retinal ganglion cells (RGC) in culture do not grow axons as rapidly as RGCs cultured from embryos. Researchers may now have identified a developmental switch that limits the ability of older RGCs to grow axons. They compared growth responses of cultured RGCs from embryonic rats and newborn rats using a wide range of conditions, neurotrophic agents and other factors, and different substrates. None of these condi-

tions enhanced the ability of RGCs from newborn rats to accelerate the growth of their axons. These results suggest that the ability of neurons to grow axons may in part be due to an intrinsic factor and not dependent on factors in the microenvironment.

The scientists next evaluated whether the change in growth rate could be signaled by other retinal cells. Additional co-culture experiments revealed that contact with developing amacrine cells signal RGCs to switch to a dendritic growth mode. The contact between RGCs and amacrine cells not only stimulated the growth of dendrites, but also impaired the growth of axons. If this signal remains in effect long after development is finished it could suppress axonal regeneration in the adult. The present work suggests that a clearer understanding of the developmental switch from axonal to dendritic growth may be a key factor in CNS regeneration. Also, the role amacrine cells play in regulating RGC growth underscores the importance that interneurons may play in suppression of CNS regeneration<sup>116</sup>. The challenge for future work remains to discover the signals that switch neurons back to the axonal growth mode.



## Neural Development

For the visual system to function properly, it must be assembled correctly during development. Similarly, for it to be repaired after damage from disease or injury, we must understand how this assembly occurs. Development involves a complex series of steps. A detailed understanding these steps and the factors that control them will shed new light on disorders and diseases that result from developmental errors and may offer clues to help develop new therapies for other neurologic diseases or conditions.

### STRABISMUS AND NEURAL DEVELOPMENT

Strabismus is a common eye disorder resulting in improper alignment of the eyes. Although strabismus has a clear tendency to run in families, the underlying genetic mechanisms involved in its pathogenesis are poorly understood. This stems, at least in part, from the variety of different forms of strabismus. There are, however, rare forms of strabismus that are inherited as classic genetic disorders, and are more approachable using current genetic techniques. Uncovering the genetic basis of these rare disorders has the potential to provide insight into the pathogenesis of more common forms of strabismus and to provide a foundation for the development of new interventions and treatments. This year, NEI-funded clinician scientists have identified two genes, KIF21A and ROBO3, which are altered in rare, genetic forms of strabismus<sup>117,118</sup>. In both cases, strabismus appears to result from developmental defects in neuronal axons that are involved in sending impulses to extraocular muscles. Further study will help target the exact role of these genes in neuronal development and may contribute insight into other forms of strabismus. Additionally, these genes may contribute toward our understanding of repairing motor neuron circuits.

### CONTROL OF EYE DEVELOPMENT

Development of the vertebrate eye is controlled by specific genes that operate in a hierarchy of expression. Some of these genes have been identified as “master controls.” In *Drosophila*, the fruit fly, loss of any one master control gene results in the failure to form an eye, while the misexpression of any of these genes is sufficient to form an eye in aberrant body locations. One of these *Drosophila* master genes, called *eyeless*, is similar to a human gene, *Pax-6*. *Pax-6* mutations result in *aniridia*, a congenital malformation of the eye associated with improper development of the iris and with the formation of cataracts. *Pax-6* and *eyeless* genes are found in other embryonic tissues, and they are crucial to the formation of other organ systems, such as the nose and antenna. In an effort to understand the function of master genes and the factors, which turn on each tissue-specific developmental program, NEI-supported scientists recently identified two signaling pathway receptors in *Drosophila* that act before the *eyeless* gene to specify eye formation<sup>119</sup>. One is the transmembrane receptor Notch that promotes eye formation. The second is the epidermal growth factor (EGF) receptor that blocks eye formation in favor of antennae. These findings are the first to suggest a mechanism of global control of eye development. Continuation of this line of research is essential to the understanding of the developmental hierarchy controlling ocular devel-





opment and will enhance our understanding of the molecular basis of congenital diseases of the eye.

#### **SLEEP AND BRAIN DEVELOPMENT**

Recent research on the developing visual system in animals has provided direct evidence that sleep in early life plays a crucial role in brain development<sup>120</sup>. In normal animals the numbers of cortical neurons dominated by inputs from each eye are roughly equivalent. Neurons receiving input from the same eye are grouped in aggregates in the visual cortex called ocular dominance columns. NEI-sponsored researchers used monocular deprivation (MD) in animal models, i.e., temporarily blocking the visual input to one eye, to develop an assay for the effects of sleep on neural plasticity. In a control group, experimental measurements of ocular dominance in the cortical surface were done after a standard MD treatment. Experimental groups were given one of three different treatments for a six-hour period following MD. One group was allowed to sleep at will in total darkness, a second group was kept awake in total darkness, and a third group was kept awake in the light. The control animals showed the expected shift in optical dominance toward the open eye. Sleep enhanced the effects of MD on visual cortical responses, but wakefulness, even in complete darkness, did not do so. The researchers theorized that sleep is a period of low sensory input during which the brain consolidates events of recently acquired tasks and that during development, sleep allows the consolidation of changes in ocular dominance evoked by short-term visual experience. Sleep

deprivation prevents consolidation of the visual experience and appears to allow accumulated changes to reverse. **The results provide the first direct evidence that sleep and sleep deprivation modify experience-dependent changes in the brain, and also suggest that synaptic circuits are modified during sleep.** Additional research exploring the mechanisms underlying sleep may provide a clearer understanding of the function of sleep.

#### **VISUAL SYSTEM PLASTICITY**

Ocular dominance columns are columns of nerve cells in the visual cortex that respond to visual input and activity from one eye or the other in binocular animals. In studies of visual system plasticity (its ability to be molded by visual input) changes in the ocular dominance columns in the visual cortex are a hallmark indicator of plastic changes during development. The long standing belief has been that ocular dominance columns emerge de novo during development from an initial state where visual inputs from the part of the brain known as the lateral geniculate nucleus (LGN), representing the two eyes, change from an overlapping representation to separate columnar aggregates each representing the input from one eye. This process is believed to take place during a limited period in development called the critical period. Experimental observations of this organization were made using injections of nerve tracers that were restricted to single layers of the LGN, with each layer of the LGN receiving its input from one eye<sup>121</sup>. However, interpreting these observations are complicated by the fact

that the presence of a continuous band of label in the young cortex could either be due the absence of segregated ocular dominance columns, or due to spillover to more than one layer of the LGN.

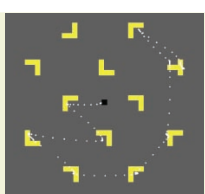
In a recent study, scientists showed that ocular dominance columns in the visual cortex of the ferret appear long before the columns can be modified by visual experience during brain development. The use of the ferret as an experimental model was critical for this new observation. The visual system of the ferret is at a much earlier stage of development at birth than the cat, commonly used for these studies. Unlike the cat, the projection of the nerve processes from the LGN to the visual cortex develop after birth in the ferret, allowing studies that would otherwise be difficult. Tracer injections confined to individual LGN layers produced clearly segregated patches of labeled cells in the developing cortex as early as postnatal days 16 to 18 in the ferret. Projections from restricted injections to both layers of the LGN gave rise to separate labeled patches in the cortex at this early stage of development in the ferret. The labeled cortical patches have all the characteristics of ocular dominance columns observed in the adult.

To further test the effects of imbalanced inputs of visual activity on ocular dominance columns, monocular enucleations were done on young animals. In all cases, the patchy cortical labeling arising from injections in the LGN persisted. These observations show that ocular dominance columns appear much earlier than previously thought, and at a much earlier stage of visual



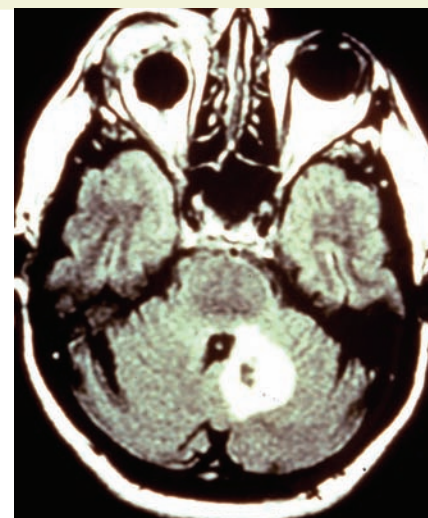
cortical development. Earlier studies on the monkey suggest a similar finding. Newborn monkeys have ocular dominance columns very similar to those in the adult. Ocular dominance columns appear to be established before they can be modified by visual experience, or put another way, the plastic changes associated with visual experience during the critical period act on pre-existing cortical columns. This new research suggests that neural activity is not required for the establishment of cortical columns; instead molecular cues guide their formation, although neural activity clearly modifies them later during the critical period. These results may also suggest that the establishment and plasticity associated with ocular dominance columns are at different stages of visual system development.





## Visual Neuroscience

Vision involves a series of highly complex neural processes that begin when light images enter the eye and fall on the retina and continue until those images are perceived in all of their detail, depth, and color. These neural processes ensure the images remain on the retina through purposeful targeted movement of the eyes or by searching and scanning movements and are focused and transmitted for processing through intricate and well-defined pathways. Progress in understanding the processes involved in visual neuroscience often has implications for other fields of neuroscience.



### NEURAL BASIS OF DECISION-MAKING

In the study of the neural basis of decision-making, vision scientists and sensory physiologists traditionally emphasize the effects of sensory stimuli on the outcome of the decision process. Psychologists and economists, however, have long known that one of the most important determinants of the decisions that humans and animals make is the value, or what economists might call the utility, of the options available to them. These impressions develop over time based upon our experience acting in the world and observing the consequences of those actions. How then, is such abstract knowledge about the value of alternatives represented in the brain? To study this phenomenon, NEI funded scientists studied a visual area of the brain of monkeys called the lateral intraparietal area (LIP)<sup>122</sup>. In these studies, monkeys were trained to play a simple video game in which they had to choose between a red and a green circle that appeared on a computer display. Sometimes the monkey was rewarded for making

a choice with something it valued, a squirt of fruit juice, but most of the time it received no reward. The important feature of this game was that choices of the red or green circle resulted in juice rewards with different frequencies that changed unpredictably over time. Remarkably, the investigators found that individual nerve cells in the LIP retain the relative value of the two options from one round of tests to the next, suggesting that these very cells of the visual system participate in encoding the brain's representation of value. Studying how brains actually represent and act on value will provide insights on how humans make decisions. Moreover, many psychiatric disorders are characterized by a disruption in one's ability to correctly value the options that are available. This is very clear in the case of addictive behavior but is also present in more subtle ways in other disorders such as depression and schizophrenia.

### NEURONAL SPECIALIZATION

When we look at a three-dimensional scene, the eyes fixate on one object at a certain depth. Images of that object project onto corresponding points of the retinas of both eyes. However, other objects within the image at different depths project to non-corresponding points of the retinas. These points create a binocular disparity; in effect, two different retinal images. These locations are related by a displacement along a horizontal axis, because the eyes are separated horizontally. Thus, finely spaced measures of horizontal disparity are required to detect variations in depth within the scene. It therefore seems natural to assume that horizontal disparities would be encoded by brain cells selective for binocular disparity. However, current understanding of neurons in the primary visual cortex suggests that different neurons signal disparities along many different axes, called their preferred monocular orientation. In the past, disparity-selective neurons were tested with disparities applied along only one axis. Such tests, however, cannot reveal whether or not neuronal responses are specialized to exploit the horizontal bias of



naturally occurring disparities. To better evaluate neuronal response, scientists explored responses of single neurons to disparities applied along several axes, using a visual stimulus that is itself not oriented<sup>123</sup>. Most neurons tested in this way modulated their firing rate over a wider range of horizontal disparities than vertical disparities, even if their preferred monocular orientation was not horizontal. This represents a specialization for the types of horizontal disparities caused by objects at different depths. This study demonstrates that early processing is more complex than previously envisaged. Recent evidence indicates that activity in disparity-selective neurons of primary visual cortex does not directly support depth perception. This study provides the strongest evidence to date that these neurons nevertheless show a specialization for horizontal disparities, and thus may play a role in depth perception.

### **VISUAL RESPONSES**

The ability to perceive changes in a visual scene requires that the visual system be able to detect or resolve the changes in both space and time. Therefore, one of the main functions of the retina is to separate visual information into these spatial and temporal components. Retinal photoreceptor cells, which capture light, are physically separated within the retina, and this separation accounts for considerable spatial resolution. However, photoreceptor light responses are uniform in their time course; and only a single neurotransmitter substance, glutamate, appears to be released by photoreceptors. Thus, temporal resolution must occur somewhere else within

the millions of neurons in the retina and brain that comprise the visual system. Several different types of specialized neurons called bipolar cell are known to receive neural impulses from the photoreceptors. Scientists attempted to determine where temporal resolution occurred by making measurements of electrical activity inside of single, distinct types of bipolar cell and found that each distinct type of neuron uses a different and distinct receptor subtype to bind the glutamate released by the photoreceptors<sup>124</sup>. Each glutamate receptor subtype responds with a different and unique time course, shaping the bipolar cell's subsequent response to glutamate released by the photoreceptors. Thus, diverse glutamate receptor subtypes with different functional properties begin the process of temporal resolution at the visual system's first synapse, the point at which neural impulses are passed between neurons. Additional research may help determine whether different glutamate receptor subtypes are associated with different morphological types of synapses at photoreceptors.

### **COLOR PROCESSING**

Perceptually, we use color information in the visual environment to discriminate objects by their hue and to identify color boundaries. Color information is encoded as electrical signals in the retina of the eye. Initially, this information is transmitted to two central structures in the brain, the lateral geniculate nucleus (LGN) in the thalamus, and then to the primary visual cortex (V1) in the brain. Many additional neuronal connections are made from the V1 in the brain to fully

process this information further. The retina and the LGN have nerve cell populations sensitive to color modulation, but the role of the V1 in visual processing has been unclear until now. The accepted view has been that color processing occurs upstream in higher visual cortical areas and that the cells in the V1 neurons are generally unresponsive to color. Recent work has reevaluated color processing in V1 by studying single neuron responses to color patterns and luminance<sup>125</sup>. These studies have found that many neurons respond robustly to color signals in V1, both to luminance and to preferred color. Contrary to the prevailing view, the primary visual cortex appears to have a crucial role in how we perceive our rich, colorful environment.





### VISUAL AND AUDITORY INFORMATION

Many objects in the real world present multiple sensory attributes. For example, an object may both reflect light and emit sound. The brain is able to determine that both the sound and the light originate from the same object, even though neural processing of spatial information by the visual and auditory systems is very different. Visual space is encoded at the level of the retina and must initially be represented in an eye-centered reference frame. In the auditory system, the location of a sound source is deduced from differences in sound arrival time and pressure level across the two ears, and is initially represented in a head-centered reference frame. At some point in the nervous system, these two reference frames must be aligned in order to create a unified percept of the single object. Recently, scientists have directly measured the responses of single neurons to noise stimuli in monkeys which were looking to the left, to the right, or straight-ahead<sup>126</sup>. The results indicated that eye position modulates the responses of neurons to sound. At the level of the inferior colliculus, where auditory stimuli converge and are then relayed to the thalamus, auditory coordinates have

already been transformed towards an eye-centered frame of reference. Why should auditory information be converted into an eye-centered reference frame? The answer may be that it is critical to be able to compare visual and auditory information.

**Localizing sounds is more difficult than localizing visual stimuli, and it appears that the brain relies on visual signals to help interpret auditory inputs.** Thus, vision may help provide the necessary feedback for auditory learning to take place.

### VISUAL AWARENESS

Of all human senses, vision is perhaps the most dominant in shaping our perceptions of the world. Yet little is known about the nature of the neural processing that allows one to know that one has seen a particular object. Two hypotheses have been postulated about how brain activity mediates such “visual awareness”. There might be a class of neurons or neural pathways whose activity mediates awareness. Alternatively, awareness might be the result of specific forms of neuronal activity, such as synchronous discharges or spike rate modulation. Scientists have recently used transcranial magnetic stimulation (TMS) to probe signaling in the brain of normal, awake humans during perception of moving objects<sup>127</sup>. They found

that the action of feedback modulation from area V5 to V1 was early and critical for awareness of motion. Thus, there do not appear to be “awareness-dedicated neurons”. Instead, awareness appears to be modulated by perceptual context, dependent upon backprojections from higher visual areas.

### VISUAL ATTENTION

As we look out our window, we think we see the whole scene but actually we do not. Instead our eyes move in rapid, successive steps, called saccades, from one part of the scene to another so that we get successive little snapshots, not the whole picture. Saccadic eye movements have long been something of a mystery in that they would seem to undermine visual stability because they move the image falling on the retina several times per second. However, previous experiments have shown that attention to specific features in the visual scene is critical for our stable perception despite these continual image shifts. Researchers have hypothesized that a region of the brain, called the superior colliculus, which generates saccadic eye movements, also contributes to the directed attention necessary for enhanced visual processing. In designing experiments to uncover the processes underlying the shifts in visual attention, researchers developed a task in which humans and monkeys have great difficulty seeing dramatic changes in a visual scene unless attention is drawn to the change. They next introduced the monkeys to a visual scene and stimulated the superior colliculus with weak electric currents in order to artificially direct



attention to the change. The researchers found that activation of the superior colliculus enabled the monkeys to see the change just as if the animals had attended to it on their own<sup>128</sup>. In this way, researchers could mimic shifts of visual attention by direct activation of the brain. These experiments therefore show that the superior colliculus is the region of the brain that generates eye movements to different regions of the visual field. It also contributes to the attention being directed to the same part of the field, and provides a first step towards understanding the circuits in the brain that underlie visual attention. This study opens the way to a better understanding of visual attention and how perception is affected when there are deficits in shifts of attention.

#### **REROUTING SENSORY NERVES**

Using an animal model, NEI-supported researchers have approached the question of how our brains organize information from our senses during development<sup>129,130</sup>. The approach involves surgically manipulating the sensory nerves that go to sensory centers of the brain. Nerves from the eye are misrouted from their usual target, the primary visual center or visual cortex, to the hearing center or auditory cortex, which has been deprived of its normal auditory input. Work with this model demonstrated that the “rewiring” procedure resulted in the development of a functional visual cortex in a part of the brain that was otherwise destined to become the auditory cortex. In these animals, the rewired visual cortex had the organization that is found in a normally wired visual cortex, and

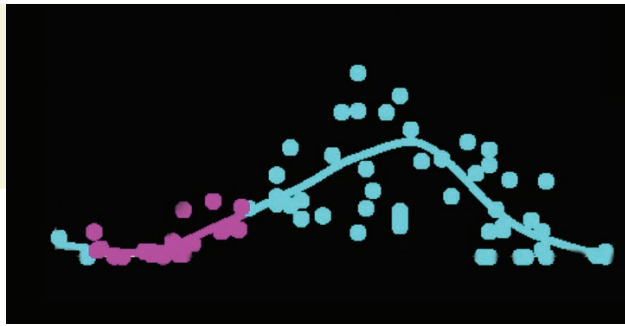
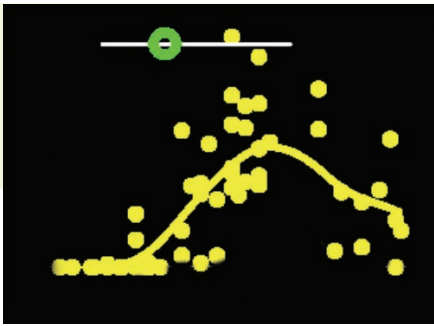
the nerve cells were taken over by the visual input. These experiments suggested that the organization and response characteristics of different regions of the brain can be shaped by the activity of nerve cells arising from the sensory input; in this case visual vs. auditory inputs.

Behavioral studies on rewired animals show that they respond appropriately to visual stimuli arising from the activity of neural circuits in the rewired centers of their brains. These animals “see” with their auditory cortex. These observations suggest that the development of brain organization is dependent on the input activity and that the sources of inputs to the brain play a significant role in determining perception and brain function. Studies of this type give us a better understanding of the development of the brain and its visual center. This information will serve as a foundation for future research on normal development, as well as abnormal development and trauma that can cause visual disorders.

#### **VISUALIZING STRUCTURE AND FUNCTION OF NEURAL TISSUE**

A long sought goal in neuroscience has been to visualize large assemblies of living nerve cells in the brain while they are active with sub-millisecond time resolution. However, imaging of the cerebral cortex has been limited to observing single neurons painstakingly labeled with calcium indicators that signal physiological activity. A recent advance in cell labeling now makes it possible to study the functional activity of thousands of neurons at once. Combining this labeling technique with a relatively

new imaging technology called two-photon microscopy, vision researchers have developed a powerful new approach to visualize the structure and function of neural tissue<sup>131</sup>. This approach offers great sensitivity, allowing researchers to see the waxing and waning of discrete neuronal responses that code for visual stimuli. One can examine all the cells present at one depth or create three-dimensional maps by imaging the cortex at multiple depths. This approach provides an unprecedented insight into the functional organization of how we process sensory information. Gaining an understanding of the circuits underlying cortical function is possibly one of the most difficult and important challenges in neuroscience. This knowledge holds great clinical importance as well. The neurological and psychiatric diseases with perhaps the largest impact on public health—Alzheimer’s disease, stroke, epilepsy, depression, and schizophrenia—are all disorders of cortical function. The application of two-photon microscopy makes it possible to study the entire cerebral cortex with unprecedented detail. Combined with advances in functional neural staining, it should also become the simplest and least invasive way to study physiological processes in normal brains and in models of neurological disease.



## Eye Movement

The study of eye movements is an essential complement to studies of vision. Because they are the most tangible output of our visual system, eye movements provide an ideal opportunity for understanding the natural consequences of visual processing. Disorders of eye movement are common symptoms of underlying neurological disease and a major cause of vision loss. Understanding oculomotor function and visual-oculomotor processing is a key step toward improved treatment of eye movement disorders like strabismus and nystagmus.

### MONITORING EYE MOVEMENT

It is essential to keep track of our own eye movements both for planning sequential movements and for maintaining stable vision despite the sudden retinal shifts caused by the eye movements. A mechanism within the brain that has been hypothesized to perform this vital function is known as a corollary discharge, that is, a copy or corollary of the neuronal commands that the brain sends out to the muscles to produce movement. Using awake, behaving monkeys as an animal model of the human visual and eye movement system, scientists have studied neurons in a pathway from the brainstem to the cerebral cortex that could convey this corollary discharge<sup>132</sup>. They found that these neurons showed the characteristics expected of a corollary discharge: first the neurons carry information that an eye movement is about to occur, second inactivating these neurons impairs the sequential eye movements dependent upon a corollary discharge and third such inactivation did not affect single eye movements that do not depend on a corollary discharge. These results identify for the first time in the primate visual system neurons that convey a corollary discharge signal. Additional work is under way to determine whether the corollary discharge signals described also are used to produce stable visual perception.



### **COORDINATED EYE MOVEMENT**

Ocular saccades are coordinated movements of the eyes. Saccades occur, for example, when we are reading, watching TV, or scanning the environment. This coordinated movement is accomplished primarily by two pairs of extraocular muscles that are attached to the globe. In visual disorders such as strabismus, the two eyes do not function as a unit and the result is a loss of binocular vision. Surgical and chemical approaches to correcting strabismus act by affecting the extraocular muscles. These treatments are often effective but may have unintended consequences. Therefore, a better understanding of the function of the extraocular muscles could lead to improvements in therapy.

An NEI grantee recently developed the “active pulley hypothesis” to explain extraocular eye movement, using Magnetic Resonance Imaging (MRI)<sup>133</sup>. Data from normal subjects has permitted a high-resolution picture of the globe and associated extraocular muscle and connective tissue. The data clearly show these muscles have different insertion points, either on the sclera (outer white covering) of the eye or on connective tissue in the orbit or socket of the eye. The identification of this organization of the muscle fibers has important implications for mathematical models of rotation properties in the eye and with further research has the potential to improve surgical approaches to the treatment of strabismus.

### **REWARD-ORIENTED EYE MOVEMENT**

Within the brain, a neuronal network known as the basal ganglia has previously been shown to control behavior. Studies by intramural scientists at the NEI indicate that the basal ganglia also modify saccadic eye movement. Saccadic eye movements are rapid eye movements that redirect one's line of sight, allowing the eyes to fix on an object or adjust their vision. With this new insight, researchers have been working to identify where in the visual system saccadic eye movements originate. Because of their proximity to caudate nucleus (CD) projection neurons, which are a major origin of eye movement signals in the basal ganglia, dopaminergic (DA) neurons (which release the neurotransmitter dopamine) have been proposed as a candidate neuron for the origination of reward-related input. To study how motivational signals modulate motor signals in the basal ganglia, NEI intramural scientists examined activity of DA neurons and CD projection neurons in monkeys<sup>134</sup>. The NEI researchers devised a visual test, called a one-direction-rewarded task, in which a visual target is presented at random positions, but only one position is associated with a big reward. In three of four monkeys studied, DA neurons responded with excitation to a reward-indicating cue and with inhibition to a no-reward-indicating cue. These results suggest that DA neurons modulate CD neurons to initiate saccadic eye movement in a reward-related manner. It is thought that the basal ganglia is affected in some neurologic diseases, such as Parkinson's, schizophrenia, and autism. Further work to elucidate the role of DA and CD neurons in health and disease could lead to important new directions in understanding neurologic illnesses.





## Refractive Error

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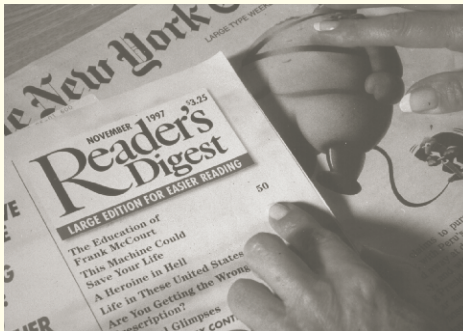
Blurred vision from refractive error such as nearsightedness (myopia) and farsightedness (hyperopia) can be relieved in most cases with eye glasses, contact lenses, or refractive surgery. Nevertheless, the high prevalence of refractive errors and the costs of correction make these conditions a substantial public health and economic problem in many parts of the world.

### THE PREVALENCE OF REFRACTIVE ERRORS

The prevalence of refractive error in the United States has not been evaluated since the early 1970s. A recent NEI sponsored study published prevalence rates for refractive error by combining data from large, high-quality, population-based eye surveys<sup>135</sup>. Based on these data, researchers estimate that refractive errors affect 42.2 million (35.3 percent) of Americans 40 years or older.

### DEVELOPMENT OF MYOPIA

About 25 percent of the adult population of the United States is nearsighted (myopic). In the most serious cases, myopia can lead to retinal detachment, glaucoma, amblyopia, and vision loss. At present there is no definitive treatment or cure for myopia. Corrective lenses and corneal surgeries merely compensate for the condition, but these treatments do not affect the underlying predisposition to myopia. The cost each year to our society for eye examinations and corrective treatment for myopia is enormous. If we could better understand the condition and develop methods to prevent myopia, the burden of this chronic condition could be lessened. **Recent animal model studies of myopia have found that the eye grows in length when images are not properly focused on the retina**<sup>136</sup>. This change occurs through a complex cascade of regulatory mechanisms and intercellular signals that originate in amacrine cells, a class of retinal neurons. These signals then spur the growth of the sclera, the outer white portion of the eye that forms the globe. It is this lengthening of the eye that further exacerbates distance vision and leads to the above aforementioned complications. Additional research identifying the specific factors that control eye growth will have significant implications for preventing or treating myopia.



## Advances in Low Vision and Blindness Rehabilitation Research

CURRENT ESTIMATES of the number of people who are visually impaired vary greatly depending on the source and method of measurement, as well as by inclusion criteria applied. Conservative estimates suggest that there are at least 3.5 to 5 million Americans who are visually impaired, and more than 1 million of these are legally blind. The leading causes of visual impairment are diseases that are common among the elderly, including age related macular degeneration, glaucoma, diabetic retinopathy, cataract, and optic nerve atrophy. Vision researchers have worked to enrich the knowledge base in both the research and clinical communities that will assist people with low vision and blindness. Their efforts have led to improvement in quality of life for visually disabled people.



Students try to perform an activity with simulated impaired vision.

### SENSORY SUBSTITUTION

Visual impairment is a major public health challenge facing this country. An important approach in overcoming this disability is the development of rehabilitation strategies that rely on other senses such as the tactile nature underlying the Braille reading system. In order to maximize and guide the effective development of these approaches, research is underway to determine the extent that other senses can be used to compensate for limited vision. **NEI-supported investigators are studying how well visual areas (occipital cortex) of the brain can be utilized by other sensory inputs, like touch, as a means for sensory substitution<sup>137</sup>.** The researchers found that the occipital cortex is involved in tactile information processing that involves discerning spatial relationships. Furthermore, the ability of the visual cortex to aide in discriminating spatial relationships is not lost in congenitally blind subjects. Such information may be of use in developing rehabilitation technologies for the blind that channel the remaining abilities of the occipital cortex through other senses.

### IMPAIRED SIGHT AND THE BRAIN

Until now it has been unclear how the part of the brain that processes central vision is affected by the lack of input from the retina in diseases like macular degeneration (MD). To better understand this question, researchers studied the visual cortex of patients who had MD for more than 20 years using a non-invasive imaging technique known as functional magnetic resonance imaging. Despite the extensive damage to the central retina, the investigators found that the part of the brain that would normally only respond to central visual information was now responding to peripheral visual information<sup>138</sup>. These findings will allow researchers to address many questions that are important in developing improved rehabilitation strategies, or in creating therapies and prosthetic devices that restore central visual function. For example, do patients with MD develop better peripheral vision than normally sighted people? How quickly does this brain reorganization occur? Is it more prevalent with early or late onset forms of MD? What are the biological mechanisms that promote these changes? Answers to these questions will lead to a better understanding of how modifiable the human brain is, and will give greater insight into the consequences of MD and the prospects for rehabilitation and treatment.



### **RESTORATION OF VISION**

A team of NEI-supported visual scientists recently studied the rare case of sight restoration in an individual who had been blind since childhood, a period of almost 40 years<sup>139</sup>. Although he had lost one eye and the sight in the other in an accident when he was three years old, the vision in his remaining eye was recently restored through a corneal stem cell transplant. Because his vision was severely deprived during a large part of the critical period of visual development that normally ends at age eight, scientists were interested in determining what qualities of vision remained or what could be re-established. Researchers found that he could indeed see, but the ability of his brain to integrate and understand what he sees was far from normal. The results of this study indicate that certain properties of vision, such as perception of color, shape, and motion, are established early in development and can survive to some degree even during an extended period of visual deprivation. Other complex features of vision, however, were absent or significantly reduced including the perception of complex objects, like faces or text; perception of the texture of a complex scene; perception in three dimensions; and many other features that constitute vision. Either the complex features of vision are not established early in visual development, or if they are, they need to be sustained by visual input. This research has provided a unique

opportunity to examine how the brain adapts during a long period of visual deprivation and to increase our understanding of the critical period. Future research in this area will be vital to full restoration of vision in those people who regain vision subsequent to its loss during the critical period.

### **DEVELOPMENT OF WAYFINDING SYSTEMS**

A major goal of blindness rehabilitation programs is the development of wayfinding systems that would allow the visually impaired to navigate their world without assistance. Such systems could potentially allow for truly independent mobility. These systems require a solid understanding of the cognitive requirements that underlie the effective use of navigation systems by the visually impaired. Recent work has shown that designers of wayfinding systems can use the mind's ability to use spatial language to update internal mental images or to map a location or object relative to the body's location<sup>140</sup>. Spatial language describes the location of an object relative to the location of a person's position (e.g. "a chair at 3 o'clock").

Vision researchers studied the role of spatial updating by experimental subjects as they moved using spatial language cues. Spatial updating refers to a person's ability, while moving, to update the location of a target previously perceived while stationary. The researchers were interested in knowing if a subject could navigate to a target using spatial language and updating as effectively as they could using auditory cues that originate from

the target source. Both blindfolded, normally-sighted subjects and blind subjects were used for these studies. All participants were tested on their ability to walk toward a target location after being cued either by auditory stimulus from a loudspeaker or by receiving spatial directions from an investigator. Two results emerged from these studies. The first result suggests that once a subject formed a spatial image it stayed fixed in the environment. The second suggests that once a mental image of a location is formed one can update the representation using either mode of input. Blind subjects were equally adept at spatial updating using spatial language, leading the researchers to conclude that visual experience may not be required for the brain to develop spatial updating skills. The findings also suggest that spatial language can convey information about important off-route landmarks (e.g. phone at 3 o'clock). The results of this study will impact significantly on the development of navigation systems for the blind and visually impaired. Wayfinding systems that use spatial language may be the best approach for development of relatively simple wayfinding systems. This is important because many of the growing number of visually impaired are elderly and require system designs that are easy to use.



### **ASSISTIVE TECHNOLOGY FOR THE VISUALLY IMPAIRED**

Researchers supported by the NEI have recently created a dual-use assistive network using ordinary fluorescent light fixtures<sup>141</sup>. The Talking Lights System provides guidance and way finding information to blind users through a minor modification to the light ballast in the fixture. The modification consists of a transmitter that can carry encoded audio, textual, graphic, or computer-control information. Users of the system can receive the signal through a hand-held receiver. The receiver decodes the signal and provides assistive information about room location and direction of travel. Preliminary trials with individuals who are blind in an office/industrial environment showed that the Talking Lights System was more effective than verbal directions given by a skilled mobility specialist. There is no additional energy use and no visible flickering or change in the light. The system uses commercial fixtures, so the costs of installation and use are low.



## National Eye Health Education Program Progress

The National Eye Health Education (NEHEP) is a Congressionally-mandated program that provides public and professional education concerning the need for early detection and timely treatment of glaucoma and diabetic retinopathy and appropriate treatment for low vision. As treatments are improved or developed and reported in research journals, the NEHEP disseminates this critical information to both patient and eye health care providers to keep them abreast of the latest research findings. NEHEP also provides patients and eye health professionals with information that can help take advantage of remaining vision, when eyesight has been lost due to disease or injury. NEHEP provides the final link in the research continuum by ensuring that those who provide or need treatment for eye disease or disorders of vision have the most up-to-date information on the available therapies based on the latest NEI-supported research.



### TRAVELING EXHIBIT ON LOW VISION

In October 1999, the NEI, through its NEHEP program, launched the Low Vision Education Program to increase awareness of low vision and its impact on quality of life. This program is directed toward people with low vision, their families and friends, and the health care and service professionals who care for them. As part of this effort, the NEI has developed a mobile exhibit on low vision that is currently traveling to shopping malls and centers throughout the United States. The exhibit contains an interactive multimedia touchscreen program; provides information on low vision services and resources; and displays aids and devices that help people with low vision. The exhibit and touchscreen program explain the causes of low vision; offer personal accounts of people living with low vision; and provide a self-assessment to help people determine if they or someone they know may have low vision.

### OUTREACH TO SELECT POPULATIONS

Through the National Eye Health Education Program, the NEI has established ongoing partnerships with more than 15 national organizations that represent communities of color and numerous national organizations that conduct specific outreach to these communities. Specific outreach programs and strategies on diabetic eye disease, glaucoma, and low vision have been developed that target African Americans, Hispanics, and American Indians/Alaska Natives. These programs are based on qualitative research with the appropriate target audiences and are reflected in the development of culturally and linguistically appropriate materials. The National Eye Health Education Program also collaborates with other Federal agencies to maximize dissemination of information. These agencies include the Bureau of Primary Health Care that includes community and migrant health clinics, HHS Office of Minority Health through Take a Loved One to the Doctor Day, Administration on Aging that includes local area agencies on aging and tribal networks, CDC that coordinates the state diabetes prevention and control programs, and CMS that reaches African Americans and people with diabetes who are at higher risk for glaucoma. The NEHEP is currently setting a 5-year agenda for program activities (2006-2011) with an emphasis on the development of culturally appropriate and health literate programs and materials.

### CULTURALLY SENSITIVE PUBLIC HEALTH PROGRAMS

Culturally sensitive and appropriate communications are central to the successful dissemination of public health information. The NEI, through its NEHEP program, convenes work groups and conducts focus groups to ensure the sensitivity of its education programs in addressing the needs and perspectives of minority populations. These activities have resulted in culturally appropriate educational materials for the Glaucoma Education Program, the Low Vision Program and the Diabetic Eye Disease Program, which includes the American Indian and Alaska Native Diabetic Eye Disease Outreach Program. Spanish language publications and public service announcements such as *Ojo Con Su Visión* (Watch Out for Your Vision) and patient brochures for diabetic eye disease, glaucoma and low vision.



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